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Ovarian Agenesis[1]

Pathology, Associated Clinical Symptoms and the Bearing on the Theories of Sex Differentiation

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RECENTLY Varnay, Kenyon and Koch (1) and Albright, Smith and Fraser (2) have called attention to a syndrome in which ovarian deficiency is associated with short stature. Patients with this syndrome usually have a moderate amount of pubic hair and sometimes axillary hair, although their breasts, vaginae and uteri remain infantile. In this respect they differ from hypopituitary dwarfs who do not develop sexual hair. The finding of high titers of urinary gonadotropin substantiated the hypothesis that the failure of normal sexual development was due to a primary ovarian deficiency and was not secondary to pituitary deficiency. These reports fail to shed light on the pathologic anatomy of the ovarian disturbance, although the authors pointed out the similarity of their patients to certain cases in the German literature in which pathologic studies demonstrated aplasia of the ovaries.

We are reporting on three patients with this syndrome in whom biopsy studies showed a total lack of ovarian development and shall discuss the relation of this finding to the theory of sex determination. Subsequently we reviewed the pathologic literature to determine a) whether patients with complete agenesis of the ovary were invariably of short stature; b) whether there are other types of arrested ovarian development or ovarian degeneration which may or may not be associated with stunted growth. In addition we have tabulated the clinical features of this peculiar syndrome, have discussed the relations of the endocrine glands to the sexual abnormalities and have attempted to explain the associated growth disturbances.

[1] This work was made possible by a grant from the Commonwealth Fund for the study of endocrine problems in childhood, supplemented by the John Howland Memorial Fund.

Case Reports

Patient L. M. (H. L. H. No. A 24951) was first seen November, 1941, at the age of 17 years. Both the maternal and paternal grandmothers were comparatively short, about 55 inches. All other members of the family, including the father, mother and three brothers, were of average height. The patient weighed 8½ lbs. at birth. Her first teeth erupted at 3 months and 12 teeth were present at one year. The second dentition began at about 7 years. She walked at 9 months, and learned to talk at the average time. She entered school at 6 years of age, but did poorly, failing several grades, and did not complete the 8th grade until she was 16. From earliest infancy she was very small and grew slowly. Although the mother had kept no definite record, she believed there had been an increase of about ½ inch in the previous year. Pubic hair first appeared at 14 years and increased slowly thereafter. The breasts did not develop and menstruation failed to occur.

Figure 1-A is a photograph of the patient at the age of 17 years, 10 months. At this time her measurements were as follows:

Patient		*Average for age*	*Average for height*
Height:	51.7 in.	64.0 in.	("Height-age," 9½ years)
Weight:	67 lbs.	117.5 lbs.	60 lbs.
Span:	52.7 in.	64.8 in.	51.2 in.
Upper segment: (pubis to vertex)	26.9 in.	32.1 in.	26.1 in.
Lower segment: (pubis to soles of feet)	24.8 in.	31.9 in.	26.5 in.
Ratio upper/lower:	1.08	1.01	1.02
Circumference head:	21.0 in.	21.8 in.	20.6 in.
Circumference chest:	28.5 in.	30.7 in.	24.4 in.
Circumference abdomen:	23.0 in.	25.4 in.	21.4 in.

The patient's features were those of a fairly mature, adolescent girl with well developed nose, chin and jaw. Her span was relatively long for her height, due probably to her broad shoulder girdle. On the other hand, the lower extremities were short in relation to her height. Her trunk was of boyish build with a broad chest and narrow pelvis. The arms showed an increased carrying angle at the elbow. The fingers were long and slender. Her musculature was well developed but not excessive. The subcutaneous fat was of average amount and uniformly distributed. The skin was of normal texture and showed no seborrhea or acne. The color was a little sallow but there was no abnormal pigmentation except for some freckles on the face. On the left side of the neck and on the back of the right shoulder there were two hard, irregular plaques in the skin which x-ray examination showed to be calcified. There was a moderate growth of brownish down on the forearms and legs. The dentition was well advanced, three wisdom teeth being present. The teeth appeared well formed. The *sexual development* was quite unusual. There was a moderate growth of long, dark, coarse hair over the labia and pubis and a fairly heavy growth of axillary hair. Both the labia majora and the labia minora were undeveloped and of infantile character. The clitoris was not enlarged. A finger could be introduced into the vagina. The vaginal cavity was quite large, the wall very smooth and without rugae. A small cervix was felt. A smear from the vagina showed some leucocytes and other small epithelial cells, with only a very few large epithelial cells. On rectal examination the uterus and ovaries were not felt. The breasts were entirely infantile. The nipples were very small, the areolae were only slightly pigmented and measured 2 cm. in diameter. No mammary tissue was felt and there was no padding of fat. The *osseous development* was that of a 13- to 14-year-old girl. The carpal bones and the styloid of the ulna were well developed. The sessamoid of the thumb was present. The epiphyses of the metacarpals and phalanges had not fused. At the elbow the trochlea, capitellum and head of the radius were completely fused with the shafts and the epicondyles and olecranon uniting. The head of the femur was fusing; the trochanters had not united. The secondary epiphyses of the crests of the ilium had not ossified. There was *generalized osteoporosis* most marked in the carpal and tarsal bones, the pelvis, spine and

toward the ends of the long bones. The skull and sella turcica appeared normal. There was no abnormality of the cervical spine and no notching of the ribs. The *mental development* according to the Gesell test was 9 years, 4 months with an I.Q. of 63 and a base line of 7 years.

The general physical findings were otherwise normal except for hypertension. The *blood pressure* in the arms was 145/95 and in the legs 160/105. No cause for the hypertension was found. Fluoroscopy showed the heart to be of normal size and shape. There was no evidence of coarctation of the aorta. The lower poles of both kidneys were palpable. Intravenous pyelograms showed no abnormality. Ophthalmoscopic examination showed no abnormality.

Laboratory data. The urine was normal. The specific gravity varied between 1.002 and 1.030. The PSP excretion was 90% in two hours. The serum NPN was 20 mg.%; hemoglobin, 13.5 gm; R.B.C., 5.6 million; W.B.C., 9880; PMN., 52%; lymph, 48%. An intradermal tuberculin test—0.1 mg.—was negative. Serological tests for syphilis were also negative; serum calcium was 10.3 mg.%; phosphorous, 3.9 mg.%; phosphatase, 3.4 Bodansky units; serum cholesterol, 126 mg.%. The B.M.R. on one occasion was +3% and on

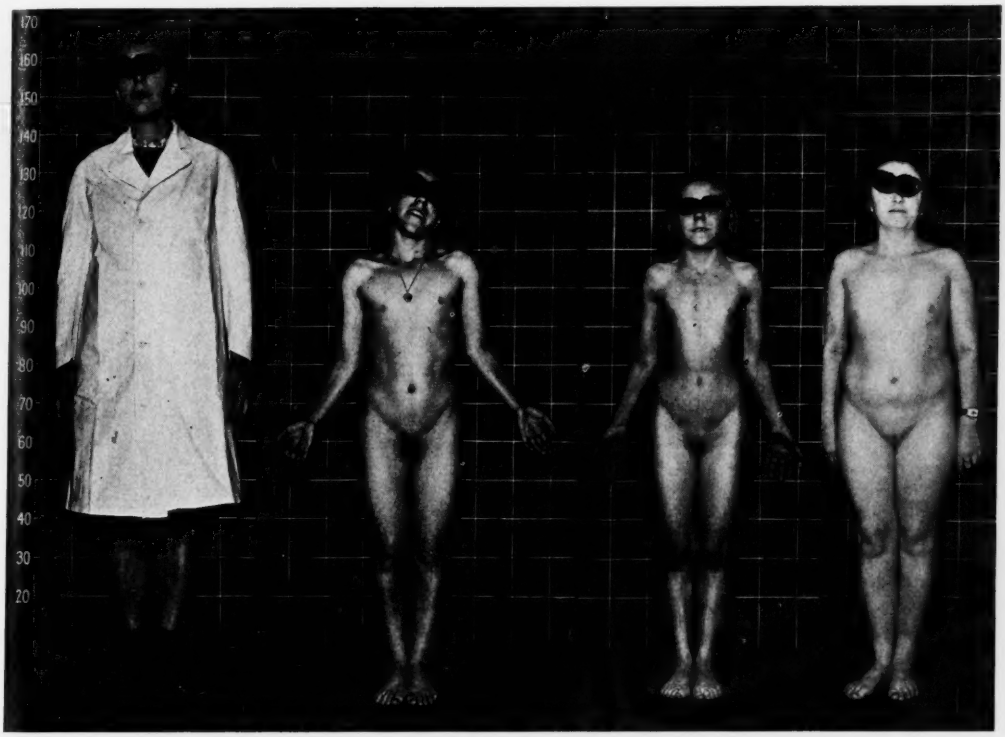

FIG. 1

Normal	A Ovarian Agenesis	B Ovarian Agenesis with Webbed Neck	C Ovarian Agenesis
18 yrs.	17 yrs. 10 mos.	19 yrs. 4 mos.	16 yrs
$\frac{\text{upper}}{\text{lower}}$ 1.01	$\frac{\text{upper}}{\text{lower}}$ 1.08	$\frac{\text{upper}}{\text{lower}}$ 1.00	$\frac{\text{upper}}{\text{lower}}$ 0.97
	Bone Age 13–14 yrs.	Bone Age 15–16 yrs.	Bone Age 14 yrs.

another, +15%. Fasting glucose was 95 mg.%. On a meat-free diet the urinary creatinine was 365 mg. per day and the creatine, 40 mg. per day. The *response to hyperglycemia* was normal on testing with the intravenous injection of 0.5 gm. glucose per kg. of body weight over a period of 30 minutes. The blood sugar dropped from 226 mg.% at the end of the injection to 86 mg.% after one hour. The *response to hypoglycemia* was normal on the intravenous injection of insulin 0.1 units per kg. body weight.

omy was performed by Dr. TeLinde. Figure 2 is a drawing of the pelvic organs which were described as follows: "The uterus could scarcely be seen but was felt in the broad ligament as a thickened structure about 1 cm. in diameter and 3 cm. long. Coming off each cornu and forming the top of the fold of the broad ligament were small fallopian tubes. The round ligaments also were readily recognized, running from the top of the uterus and leaving the abdominal cavity at the internal ring. On the

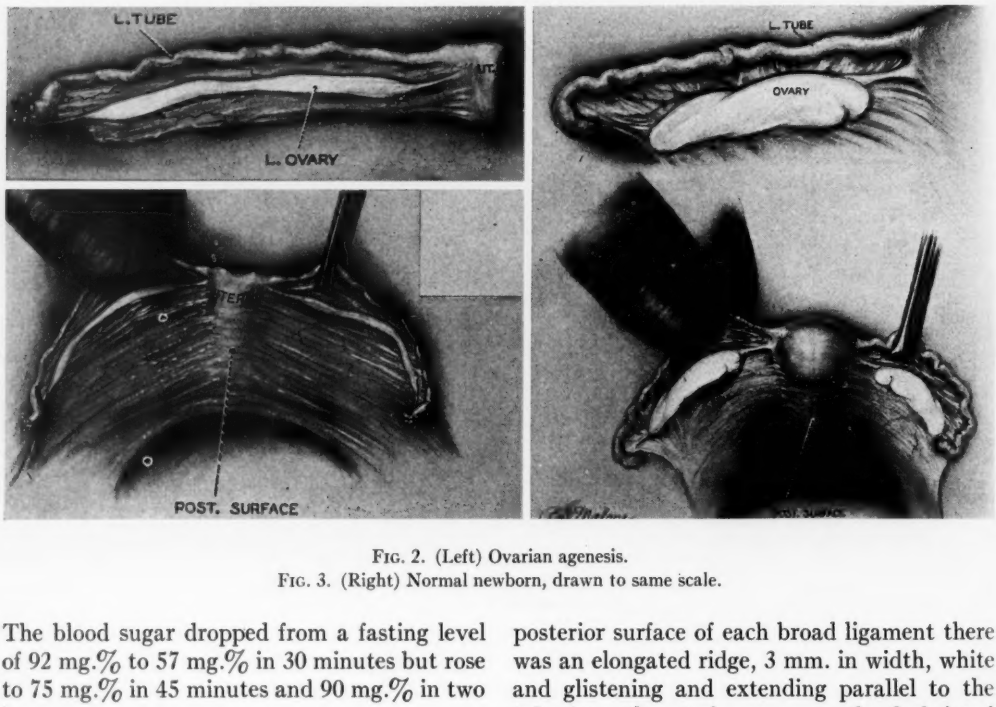

FIG. 2. (Left) Ovarian agenesis.
FIG. 3. (Right) Normal newborn, drawn to same scale.

The blood sugar dropped from a fasting level of 92 mg.% to 57 mg.% in 30 minutes but rose to 75 mg.% in 45 minutes and 90 mg.% in two hours.

Before treatment *the urinary gonadotropin* assayed by Dr. Seegar-Jones on the uterine weight of the immature rat (method of Heller and Heller, 3) showed less than 6 rat units per 24 hours. The patient was treated with estradiol-benzoate 1.66 mg. per day from June 25 to July 3. On July 28, 25 days after discontinuance of treatment, the gonadotropin assay showed 14 rat units per 24 hours. Before treatment *the output of 17-ketosteroids* was 2.8 mg. in 24 hours, whereas 25 days after treatment was discontinued, 6.1 mg. was excreted.

On January 4, 1942 an *exploratory laparot-*

posterior surface of each broad ligament there was an elongated ridge, 3 mm. in width, white and glistening and extending parallel to the tube from the uterine cornu to the fimbriated end. This ridge was considered to be the embryonic rudiment of the ovary as no other structure resembling an ovary was found. The tube and rudimentary ovary on the left side were excised and sectioned for microscopic study. Figure 3 is a drawing on the same scale of the sex organs of a normal newborn. *On microscopic examination* the tube showed the usual folds of epithelium. Only one type of columnar cell having little cytoplasm was seen. There were no definite ciliated cells or peg cells. At the site of the 'ovarian streak,' the broad ligament was definitely thickened. At

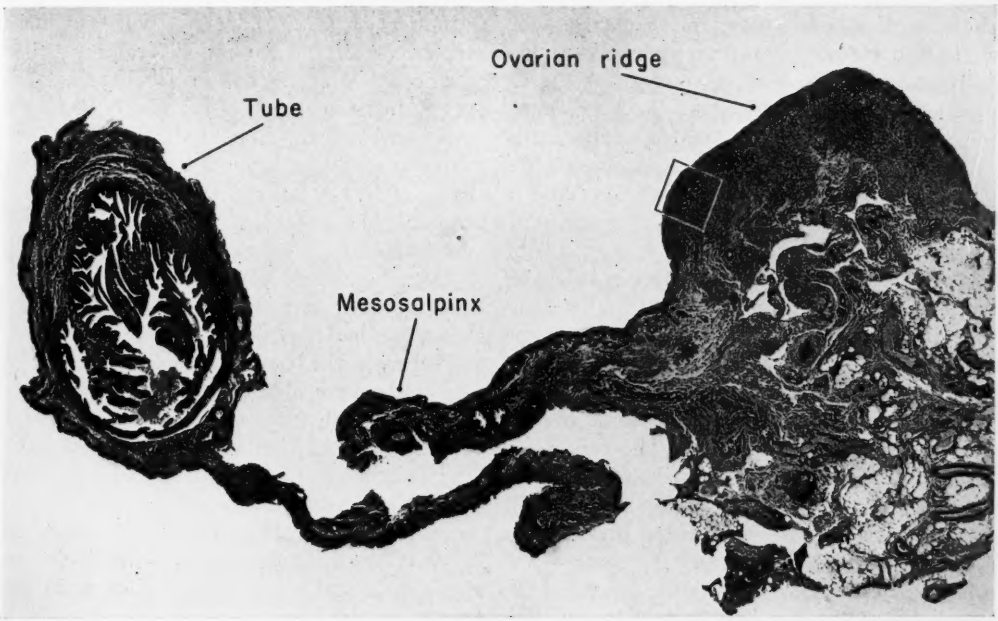

FIG. 4. Tube, mesosalpinx and ovarian ridge—Patient L. M.

this point the tissue looked exactly like ovarian stroma composed of elongated spindle-like cells arranged in whorls blending gradually into the ordinary connective tissue of the broad ligament. Over this region there was no definite layer of epithelial cells corresponding to the germinal epithelium. No follicles were found in spite of the fact that the whole ovary was cut into blocks and sectioned."

Figure 4 is a low-power microphotograph of a section taken through the tube, mesosalpinx, ovarian ridge and broad ligament. Figure 5 is a high-power microphotograph of the rudimentary ovary compared to a similar section (fig. 6) of the ovary of a normal newborn.

Treatment and Subsequent Course. In June, 1942, metabolic studies were made to determine the effect of a ten-day period of treatment with estradiol-benzoate. During August the patient was treated at home with a daily

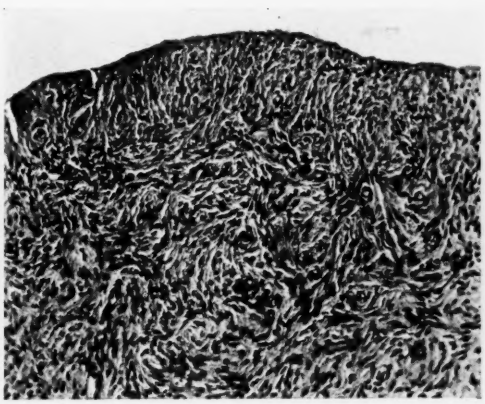

FIG. 5. Ovarian agenesis—Patient L. M.—×250.

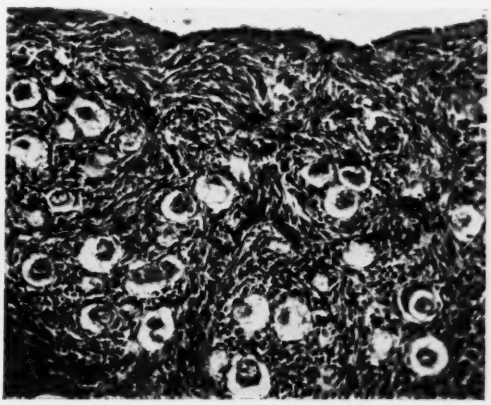

FIG. 6. Ovary of normal newborn×250.

dose of 1 mg. of stilbestrol. From September 1 to December 23 further metabolic studies were made in the hospital, comparing the effects of stilbestrol and estradiol dipropionate. Following this, she was treated continuously with a daily dose of 1 mg. of stilbestrol. In May, 1943, a few drops of blood appeared in the vagina but otherwise no bleeding occurred. Between September, 1942 and June, 1943 the patient grew 0.9 inches, reaching a height of 52.7 inches. Her weight increased 7.6 lbs. During this time no marked change in the fusion of the epiphyses occurred and there was no definite change in the density of the bones. The patient's features matured somewhat and became slightly more feminine. The contour of the trunk did not change much; the chest remained broad and the shoulders square. The most striking change was in the breasts. The nipples and areolae became prominent and deeply pigmented; the areolae measured 2.5 cm. in diameter. The breasts were conical. The mammary tissue measured about 4 cm. in diameter and was of normal consistency. The pubic, labial and axillary hair became somewhat more abundant. The labia majora were more prominent and more pigmented. The labia minora developed definitely but were still of less than adult size. The vaginal outlet admitted only one finger but the caliber of the vagina was practically normal for an adult and the mucosa showed prominent rugae. A vaginal smear showed some leucocytes and rather small epithelial cells. Very few large epithelial cells were present. The cervix was of almost normal diameter and was 2–3 cm. long. It was still impossible to palpate the uterus. It is of interest that following the exploratory laparotomy a large keloid developed in the vaginal scar and showed a deep brown pigmentation.

Patient M. H. (H. L. H. No. 39480) had been an inmate of the Rosewood Training School for Feeble-Minded Children from the age of 7 years. Little is known of her family history or early development. She weighed 5 lbs. at birth and was stunted in growth from early infancy, being 26 inches tall and weighing 11 lbs. at 12 months. She walked at 2 years but was very much retarded in her mental development. The breasts did not develop and menstruation had not occurred although a scanty growth of labial hair had appeared.

Through the courtesy of Dr. George A. Johns we were able to study this patient in January, 1940, when she was 16 years and 10 months old. At that time she was 50 inches tall (height-age $8\frac{1}{2}$ years) and had a bone age of 14 to 15 years. Her condition was followed during the next $2\frac{1}{2}$ years during which she grew 0.7 inch. There was slight increase in the labial hair and a scanty growth of pubic hair but no axillary hair appeared. The breasts did not develop.

Figure 1 B is a photograph of the patient at 19 years, 4 months. Her measurements at this time were as follows:

	Patient	*Average for age*	*Average for height*
Height:	50.7 in.	64.0 in.	("Height-age," 9 years)
Weight:	57.0 lbs.	117.5 lbs.	56.5 lbs.
Span:	53.4 in.	64.8 in.	50.1 in.
Upper segment: (pubis to vertex)	25.3 in.	32.1 in.	25.8 in.
Lower segment: (pubis to soles of feet)	25.4 in.	31.9 in.	24.9 in.
Ratio upper/lower	1.00	1.01	1.04
Circumference head:	20.0 in.	21.9 in.	20.5 in.
Circumference chest:	25.0 in.	30.9 in.	24.2 in.
Circumference abdomen:	21.2 in.	25.6 in.	21.2 in.

Her skeletal proportions were fairly normal except that the span was a little long in relation to her height. Her neck was short and showed marked webbing. X-ray plates showed fusions and spina bifida involving several of the cervical vertebrae similar to the Klippel-Feil syndrome. Neurological abnormalities such as mirror movements often associated with this defect were not demonstrated. The scapulae were flaring. There was bilateral ptosis of the eyelids. Her features were slender and rather immature but the ptosis and compensatory raising of the eyebrows caused her to have a somewhat "drawn," anxious expression. There was a moderate cubitus valgus.

Her shoulders and chest were comparatively broad and pelvis narrow, causing a boyish contour of the trunk. Her hands and fingers were slender. Subcutaneous fat was scanty and the musculature was good but not excessive. The skin was of normal texture and showed no seborrhea or abnormal pigmentation. No subcutaneous nodules or calcified plaques were felt. There was no increase of down on the trunk or extremities. The dentition was well advanced, two wisdom teeth being present. The teeth were crowded but not carious. As to *sexual development* the breasts and nipples were entirely infantile, the areolae being unpigmented and measuring only 1 cm. in diameter. No mammary tissue was palpable. There was a fairly abundant growth of light brown, long coarse hair on the labia majora but only a little fine colorless down over the mons veneris. No axillary hair was present. The labia majora were poorly developed and unpigmented. The labia minora were practically absent. The clitoris was not enlarged. The vagina was about 5 cm. in length with smooth walls. A very slight dimple could be felt representing the external os. Through the rectum the uterus was felt as a cord-like structure about 3 cm. long and 0.5 cm. in diameter. A vaginal biopsy showed prepubertal vaginal mucosa. The *osseous development* was that of a 15- to 16-year-old girl. The epiphyses at the elbows were completely united except for a slight separation of the olecranon and of the internal epicondyle. The metacarpal epiphyses were fusing but those of the phalanges were still separated from the shafts. The head and trochanters of the femurs were completely united with the shafts although the remains of the lines could be seen. The other epiphysial lines were still open although they were narrow. There was marked *generalized osteoporosis* most conspicuous in the tarsal and carpal bones and the ends of the long bones. In the left os calcis there was a clear spheroidal cyst 1.7 cm. in diameter. There was a small, poorly defined, cystic area in the cortex of the left radius and another in the ulna. No abnormality of the skull or sella turcica was noted. *Mental standardization* at the age of 16 years, 10 months, showed a mental age of 6 years, 8 months with an I.Q. of 47 and a base-line of 5 years. She was right-handed, left-eyed and left-footed.

The *blood pressure* in the right arm was 148/112 and in the left arm, 152/110. No pulse was palpable in either leg in the femoral triangle, popliteal space or dorsum of the foot. A systolic murmur was heard over the back, especially in the interscapular region. No enlargement of the heart was demonstrated by percussion or teleoroentgenogram. It was felt that these findings were diagnostic of *coarctation of the aorta* in spite of the fact that a roentgenogram did not show any characteristic changes of the aorta or notching of the ribs. No collateral circulation was demonstrated.

Laboratory Data. The hemoglobin was 13.8 gm.; R.B.C., 5.02 million; W.B.C., 9600; PMN, 76%; lymph., 18%; monocytes, 6%. Examination of the urine showed no albumin, sugar or acetone. Specific gravity varied from 1.006 to 1.026. The P.S.P. excretion was 90% in two hours. The Wassermann test was negative. Intradermal tuberculin, 0.1 mg., gave a positive reaction. The serum N.P.N. was 33 mg.% and the fasting sugar was 96 mg.%. Blood serum calcium was 10.3 mg.%; phosphorus, 4.5 mg.%; phosphatase, 7.8 Bodansky units. Serum cholesterol varied from 155 to 193 mg.%; serum sodium was 140 milliequivalents/liter. The B.M.R. was +13% and +15%. The average excretion of creatinine was 408 mg. per day; of creatine, 85 mg. per day. *The response to hyperglycemia* was normal. On testing with the intravenous injection of 0.5 gm. glucose per kg. of body weight over a period of 30 minutes, the blood sugar dropped from 328 mg.% at the end of the injection to 148 mg.% in 60 minutes and to 94 mg.% in 90 minutes. *The response to hypoglycemia* was tested by injecting crystalline insulin, 0.1 units per kg. of body weight. The blood sugar values were as follows: fasting, 82 mg.%; 20 minutes, 44 mg.%; 30 minutes, 45 mg.%; 45 minutes, 65 mg.%; 60 minutes, 75 mg.%; 90 minutes, 63 mg.%; 120 minutes, 60 mg.%. The rebound from the hypoglycemic level was considered within normal limits.

A urinary gonadotropin assay made by Dr. Seegar-Jones using the weight of the uterus of the immature rat according to the method of Heller and Heller (3) showed 85 rat units in 24 hours.

An assay of the 17-ketosteroids in the urine showed an excretion of 2.2 mg. in 24 hours. The patient was treated with estradiol-dipropionate, 5 mg. daily, for 14 days. Thirty days after discontinuing treatment the urinary 17-ketosteroids were 5.3 mg. in 24 hours.

An exploratory laparotomy was performed by Dr. TeLinde who stated, "The findings were in every way identical with those in the first patient (*L. M.*) The rudiments of the ovaries were elongated, whitish ridges on the broad ligaments parallel to and extending almost the length of the fallopian tubes. Microscopic sections (fig. 7) showed a histological picture similar to that of the first case. The tissue was composed of cells resembling ovarian stroma but

FIG. 7. Ovarian agenesis—Patient M. H.—×250.

in one area was covered with a layer of cuboidal surface epithelium such as one sees on a normal ovary. There was no evidence of follicle formation in sections cut at various levels."

Treatment and Subsequent Course. In October and November, 1942, metabolic studies were made for the purpose of comparing the effects of estradiol-dipropionate and stilbestrol. Beginning December, 1942, the patient was treated continuously with a daily dose of 1 mg. stilbestrol by mouth. She states that a little vaginal bleeding occurred in March and April, lasting about 3 days each time. Between December, 1942, and June 26, 1943, the patient did not increase at all in height but gained 7.9 lbs. in weight. The failure to grow can be explained by the fact that the epiphysial fusion was already complete at the elbows and hips, though other epiphysial lines were partly closed. In June, 1943, x-ray plates showed that no rapid change in epiphysial fusion had occurred after the institution of estrogenic therapy. Possibly the bones had become slightly denser although osteoporosis was still marked. The cysts appeared to be filling in. The patient's features and bodily contours had not changed. The most marked change was in the breasts which had become hemispherical with very dark brown areolae and nipples measuring 2 cm. in diameter and projecting like the cups of acorns. The underlying mammary tissue measured 4 cm. in diameter. As in the case of our other patient, the surgical scar had developed a prominent keloid which showed dark brown pigmentation. The pubic and labial hair had become more prominent but no axillary hair had appeared. The labia majora were deeply pigmented and fairly prominent. The labia minora had developed only slightly. The outlet admitted only one finger but the vagina was quite roomy. The vaginal mucosa was still relatively smooth. A vaginal smear showed large flat cells with small, deeply staining nuclei. The cervix was still quite small and the uterus and adnexa could not be felt.

B.J.W. (*H.L.H. No A 33191*) was first seen at the age of 16 years, November 13, 1943. The father and mother were of average height. The maternal grandfather, however, was only slightly over 5 feet tall. The patient weighed 6 lbs., 2 oz. at birth. Her first teeth erupted at 6 months. Second dentition began at 5 to 6 years. She walked at 14½ months. When she entered school at 6 years she was by far the shortest in her class and has been the smallest ever since. She has done very well in her school work. Her breasts have not developed at all. About two years ago, in January, 1942 she began to complain of cramp-like pain in the abdomen. She was treated with pills (stilbestrol?). Pain occurred about once a month (in February and March, 1942) and lasted several days. There was never any bleeding or discharge. Thereafter treatment was discontinued. For a period of at least one year prior to examination she had a little hair on the labia. She is reported to have been deaf in the left ear all of her life. Her photograph is shown in figure 1C.

	Patient	*Average for age*	*Average for height*
Height:	53.5 in.	63.5 in.	("Height-age," 10¼ years)
Weight:	88.7 lbs.	114 lbs.	65.4 lbs.
Span:	55.2 in.	64.2 in.	53.1 in.
Upper segment: (pubis to vertex)	26.3 in.	31.8 in.	26.9 in.
Lower segment: (pubis to soles of feet)	27.2 in.	31.7 in.	26.6 in.
Ratio upper/lower:	0.97	1.00	1.01
Circumference head:	19.8 in.	21.7 in.	20.7 in.
Circumference chest:	29.4 in.	30.1 in.	25.0 in.
Circumference abdomen:	26.5 in.	25.1 in.	21.8 in.

In proportion to her height, the span and lower segment were slightly longer than average. The circumference of the cranium was 2 inches less than the average for her age and 1 inch less than the average for her height. The features were those of an adolescent girl of her age. The eyes were rather closely spaced and the eyeballs were quite prominent. There was no ptosis of the lids or strabismus. There was an asymmetry of the lower portion of the face, the right side of the mandible being less developed than the left. The chin was very poorly developed and the skin of the neck receded sharply backward from the tip of the chin. The palate was highly arched rising to a point. The naso-orbital folds were rather marked, giving her an expression resembling that of patient *M.H.* There was no webbing of the neck. The shoulders and chest were broad. The subcutaneous fat was more abundant than average. The skin was normal and no subcutaneous nodules were felt. All of the deciduous teeth had been lost. Only 3 of the 12-year molars had erupted and the upper canines were absent. As to *sexual development*, there was a slight prominence in the breast region due to subcutaneous fat. The nipples were of preadolescent size but erectile. The areolae measured 2 cm, and showed but little pigmentation. No development of the mammary glands could be detected. There was no hair on the pubis or in the axillae but a small amount of coarse, brown hair was present on the labia majora. The labia majora were undeveloped. The labia minora and introitus were infantile. The clitoris was quite small. The *osseous development* was that of about 14 years. Epiphysial centers at the elbow were the only ones which had fused. There was rarefaction of the bones, most marked in the hands and feet and in the ends of the long bones. The *mental development* was normal. The general-examination findings were normal except for the *blood pressure* which was 130/100 in the arms and 134/96 in the legs. There was no evidence of coarctation of the aorta. Intravenous pyelograms showed no abnormality. An ophthalmological examination showed myopic astigmatism, mild exophthalmos and slight vertical muscle imbalance. The eye grounds and visual fields were normal.

Laboratory Data. The urine was normal. The P.S.P. excretion was 55% in two hours. The serum N.P.N. was 39 mg.%. The urea clearance and the mannitol clearance were normal. The serum calcium was 10.9 mg.%; phosphorus, 5.2 mg.%; phosphatase, 5.9 Bodansky units; and serum cholesterol, 256 mg.%. The B.M.R. on one occasion was −14% and on another, +5%. The results of a glucose tolerance test (response to hyperglycemia) and an insulin tolerance test (response to hypoglycemia) were normal.

The urinary FSH was assayed at the Massachusetts General Hospital by a modification of the Heller and Heller method. On the undialyzed specimen of urine the test result was negative for 96 mouse units per 24 hours; on a dialyzed specimen it was positive for 78 mouse units per 24 hours. The output of *17-ketosteroids* was 4.5 mg. in 24 hours on one specimen; 3.1 mg. per 24 hours, on another.

Exploratory laparotomy was performed by Dr. TeLinde. The uterus measured 3.5×11.5 cm. The tubes and ovaries were identical in appearance with those in the two other cases reported herewith. An excised tube and ovary showed the same histological structure, the ovary being composed entirely of stroma-like cells.

EMBRYOLOGY AND PATHOGENESIS

The primordium of the sex gland first makes its appearance in the 5–7 mm. human embryo on each side of the body as a thickening of the coelomic epi-

thelium known as the *germinal epithelium* to form the *genital ridges* lying on the medioventral surface of the mesonephros. This germinal epithelium consists of a large number of ordinary cuboidal or columnar epithelial cells and a few *primordial germ cells*. There is some disagreement as to whether the primordial germ cells arise elsewhere from either embryonic or extra-embryonic entoderm and later migrate to this site, or whether they are differentiated in the genital ridge directly from the germinal epithelium. The germinal epithelium proliferates as "*primary sexual cords*" extending into the underlying stroma of mesenchyme. At this time mesonephric tubules are developing from the Wolffian bodies. These become connected with the primary sex cords by means of strands of cells called the *rete cords*. Up to this stage of development, which is reached in the 20 mm. human embryo, there is no morphological difference in the gonads of the two sexes. Sex differences now appear in the further development of (a) the germinal epithelium; (b) the mesenchymal stroma; (c) the rete cords; (d) the efferent tubules of the mesonephros. In the female the germinal epithelium proliferates downward from the surface, giving rise to nests of cells which form the primordial follicles, while the theca cells and stroma probably arise from the mesenchyme. The rete cords remain as rudimentary structures in the rete ovarium, while remnants of the mesonephric tubules persist as the epoöphoron and paroöphoron. In the male the rete cords assume major importance in forming the outlet of the seminiferous tubules and the mesonephric tubules become the efferent ducts of the epididymis.

In the three cases which we have described the gonads apparently had developed but little, if at all, beyond the stage of the primitive genital ridge. The elongated, whitish body which was found lay lateral to the fallopian tubes and was therefore situated in the position in which we would expect the genital ridge to be carried by the outgrowth of mesentery which forms the broad ligament. On microscopic section there was no evidence of any proliferation of the germinal epithelium into cords or nests of cells suggesting the formation of follicles. No sex cells were seen. The structure was composed largely of stroma-like cells apparently derived from the mesenchyme. There were a few nests of cells which corresponded to the rete.

Whether primordial germ cells ever developed in the very early embryo cannot be determined from the study of our cases. It does seem fair to assume that if such cells developed elsewhere they never migrated into the genital ridges. The experiments of Reagan (4) Benoit (5) and Dantschakoff (6), who excised or destroyed by roentgen rays the areas of germ-cell formation in the very early chick embryo, show that the migration of germ cells into the genital ridge is essential for the proliferation of the germinal epithelium and the differentiation of this tissue into the gonad. In such cases (Reagan) the ridge persists as stroma cells covered by a thin layer of "germinal epithelium" apparently resembling the condition in the cases we have described. On the other hand, the work of Willier (7) and Dantschakoff (8) shows that the germ cells have no specific organizing effect upon mesoderm in locations other than the genital ridge.

The cases described here are of considerable interest because of their bearing upon the theories of sex determination. This was pointed out by Rössle and Wallart (9), who described a patient similar to ours, and by Schiller (10). In spite of the complete lack of development of their gonads, the general development of our patients was entirely along female lines. The Müllerian duct system was developed to the state of a normal 7- to 8-month, female fetus. The Wolffian duct system had entirely disappeared; no remnants of Gaertner's duct was found. Only moderate amounts of rete tissue and of epoöphoron ductules persisted in the gonadal region.

It is generally accepted that zygotic influences determine sex through the differentiation of the gonad into ovary or testis. There is less agreement concerning what determines the differentiation of the accessory sexual apparatus. Various theories have been proposed which differ in the relative importance attributed to: (a) hormones produced by the embryonic gonads; (b) zygotic factors; (c) maternal hormones. According to the "di-hormonic" theory, either female or male hormones may exert an influence so that the development of homologous ducts, the degeneration of heterologous ducts and the normal differentiation of other sexual organs are controlled by hormones elaborated in either the embryonic ovary or testis. This theory is upheld by Greene (11) on the basis of experiments in which the administration of hormones to pregnant rodents influenced the sex differentiation of their offspring. The "mon-hormonic" theory proposed by Wiesner (12) in 1935 states that only androgen can influence differentiation. It argues that without any hormone the development of

all embryos would be feminine and in males the hormone elaborated by the embryonic testis converts the differentiation to the masculine type. A modification of this theory is that the general tendency toward female development is attributable to the influence of maternal estrogen. It is difficult to concede the influence of maternal hormones on sex differentiation unless one grants also that the embryonic testes produce hormone capable of reversing the action of maternal estrogens. On the basis of studies on the opossum, in which hormones were administered and in which embryonic castration was carried out, Moore (13) opposes both these theories and comes to the conclusion that sex hormones are not produced by the embryonic gonads and that both the differentiation of the gonads and the sex pattern of duct development are determined entirely by zygotic factors. Experiments on embryonic castration intended to establish such a condition are as yet inconclusive. The various studies on the problem have been reviewed and discussed by Burns (14) and by Greene (11).

The cases of ovarian agenesis offer convincing evidence that the female differentiation of the accessory apparatus does not depend upon the production of estrogen by the embryonic gonad. Although they suggest a purely zygotic determination they do not exclude Wiesner's postulate that in the absence of androgen all sex development would be feminine, nor can the possible role of maternal estrogen be entirely excluded. For conclusive proof that sex differentiation is determined solely by zygotic factors it would be necessary to find male individuals analogous to our patients and lacking entirely in gonadal development.

In explaining the etiology of the cases which we have reported one must assume that in the zygote from which the patient arose there was some defect in the germ plasm. This defect was responsible for the failure of the embryo to develop primordial germ cells or at least for germ cells to persist in the genital ridges. This in turn led to a failure in the development of the ovaries. However, the defect did not affect the sex-controlling factors of the chromosomes because the development of the Müllerian ducts and the retrogression of the Wolffian ducts followed the normal female pattern. This suggests that separate genes may be responsible for sex determination (gonad formation) and for sex-controlled characters (duct differentiation).

REVIEW OF PATHOLOGICAL REPORTS IN THE LITERATURE

In table 1 we have collected 15 cases, in addition to the three we are reporting, in which autopsy or biopsy studies showed absent or rudimentary ovaries. In all of the patients the breasts, vaginae and uteri were infantile; menstruation had never occurred. The presence of labial, pubic or axillary hair in all but two (*Cases 4* and *14*) suggested that androgen was being produced by the adrenals.

Eleven of the patients (*Cases 1* to *11*) (including our three cases) showed a complete lack of ovarian development. There was a whitish streak representing a persistent genital ridge composed only of stroma-like cells. Histological studies showed no proliferation of the epithelial layer, no cells suggestive of germ cells and no primordial follicles. The amount of persistent mesonephric elements, namely rete tubules and epoöphoron, varied somewhat in different cases. In one patient of Pich (*Case 6*) (18) there were some large nests of cells resembling interstitial (Leydig's) cells which were probably derived from the adrenogenital ridge. Probably due to an androgenic effect exerted by these cells, this patient had a slight enlargement of the clitoris, some development of prostatic tissue, and a persistent Gaertner's duct. Of these patients who showed a complete absence of gonadal development, ten were less than 59 inches tall, while only one, the patient of Meyer (*Case 8*) (20), in whom there was a high titer of urinary gonadotropin, was of normal height—66.7 inches. We shall discuss the significance of this finding in a later section.

In four other patients (*Cases 12* to *15*) a somewhat different condition was found, suggesting that ovarian development had advanced to varying degrees before it was arrested. In Pela's (23) case the ovaries were described as "the size of orange seeds" instead of the long, slender ridges seen in our patients. However, no primordial follicles were present

and no mention is made of any proliferation of the germinal epithelium. In Tronci's (21) case, the ovaries were described as oval bodies 6×2.5 mm. in diameter. There were down-growths of germinal epithelium forming nests of cells in the stroma but no primordial follicles. In *Case 14* (Pich, 18) and *Case 15* (Sellheim, 22) development had progressed still further since fairly numerous primordial follicles were present. The cause of the arrested development cannot be determined from the pathological studies. No evidence of inflammatory or other destructive lesions was found. Since assays of the urinary gonadotropins were not made, it is impossible to decide whether or not the failure of development may have depended upon a deficiency of the pituitary gonadotropins. It is of considerable interest that in this group, only the patient of Tronci, who was 58 inches tall, was of short stature. Pich's and Sellheim's patients were of average height, while Pela's patient was 70 inches tall and had eunuchoid proportions. It is possible that there are a number of different types of hypo-ovarianism, the classification and differentiation of which we have discussed in a previous paper (33).

We have listed three other cases (*16*, *17*, *18*). In the patients of Priesel (24) and Sharpey-Shafer (25) no trace of ovary was found at autopsy but both had extensive tuberculous peritonitis. Both patients were short. The patient of Kuliga (26) was tall, of eunuchoid proportions. No ovary was found during operation for appendicitis. No deductions can made from these cases.

TABLE 1

Case No.	Author	Autopsy or biopsy	Age	Height inches	Menstruation	Breasts	Sexual Hair Pubic Pub.	Sexual Hair Axillary Axil.	Ovaries: Size & Shape	Ovaries: Proliferated germinal epithelium	Ovaries: Primordial Follicles	Ovaries: Interstitial Cells	Other Abnormalities
1	Olivet (15)	A	38	58.5	0	0	+	+	Streak	0	0	0	
2	Randerath (16)	A	61	57.0	0	0	±	±	Streak	No histological study			
3	Schürmann (17)	A	25	56.3	0	0	0	±	Streak	0	0	0	
4	Rössle (9)	A	39	52.3	0	0	0	0	Streak	0	0	0	
5	Pich #1 (18)	A	18	52.0	0	0	±	0	Streak	0	0	0	
6	Pich #2 (18)	A	49	58.7	0	0	±	0	Streak	0	0	+	Clitoris enlarged Prostate, Gaertner's duct
7	Goldwasser (19)	B	23	55.5	0	0	±	0	Streak	0	0	0	Webbed neck
8	Meyer, R. (20)	B		66.7	0	0	+	±	Streak	0	0	0	F.S.H.+
9	Wilkins & Fleischmann #1	B	17	51.7	0	0	+	++	Streak	0	0	0	F.S.H.+
10	Wilkins & Fleischmann #2	B	19	50.7	0	0	+	0	Streak	0	0	0	F.S.H.+; Webbed neck Coarctation of aorta
11	Wilkins & Fleischmann #3	B	16	53.5	0	0	Slight labial	0	Streak	0	0	0	F.S.H.+
12	Pela (23)	A	36	70.0	0	0	±	0	"Size of orange seeds"	0	0		
13	Tronci (21)	A	20	58.0	0	0	±L	0	6×2.5 mm.	+	0		
14	Pich #3 (18)	A	29	61.7	0	0	0	0	R.28×5×3 mm. L.30×4×2 mm.	+	+		Coarctation of aorta
15	Sellheim (22)	B	21	63.7	0	0	±	0	2.5×7.5		++	+	
16	Priesel (24)	A	45	52.0	0	0	±	0	None seen—Tbc. peritonitis				
17	Sharpey-Shafer (25)	A	21	55.0	0	0	±	0	None seen—Tbc. peritonitis				
18	Kuliga (26)	B	25	69.0	0	+	±	±	None seen—Appendectomy				

Review of Papers on the Clinical Features of the Syndrome of Ovarian Deficiency and Short Stature

A number of the foreign writers—particularly Rössle (9), Pich (18), Sharpey-Shafer, (25) and Tronci (21)—noted and discussed the association of stunted growth and ovarian dysplasia and Pich reviewed the cases which had been reported previously. However, our knowledge concerning the various clinical features

encountered in this syndrome is due largely to the recent papers of Varney, Kenyon and Koch (1) and Albright, Fraser and Smith (2) who based the diagnosis of *primary* ovarian deficiency on the finding of high titers of urinary gonadotropin.

In 1938, Turner (27) described seven girls from 15 to 23 years of age, who varied from 48.5 to 55 inches in height and presented a "syndrome of infantilism, congenital webbed neck and cubitus valgus." Their breasts, vaginae and uteri were infantile and menstruation had not occurred. In five of the patients a scanty growth of either labial, pubic or axillary hair was observed; but the writer did not point out the significance of this finding in distinguishing such patients from cases of dwarfism with sexual infantilism due to pituitary insufficiency. Since assays of the urinary gonadotropins were not made, there is no proof that the condition was due to primary ovarian deficiency. However, the similarity of Turner's cases to our second case, Albright's *Case 7*, and Sharpey-Shafer's case makes it probable that their pathogenicity is identical. Recently Schneider and McCullagh (28) have reported five cases of Turner's syndrome.

In 1942, Varney, Kenyon and Koch (1) described four young women with short stature and retarded sexual development who had high titers of urinary gonadotropin. In only the first two cases (*R.F.* and *A.B.*) was there total absence of ovarian function as evidenced by complete lack of breast development and menstruation. These authors pointed out the differences between these patients and cases of sexual infantilism due to hypopituitarism and noted their relationship to the cases in the foreign literature in which ovarian aplasia had been demonstrated at autopsy. In the same year, Albright, Smith and Fraser (2) reported 11 cases of "primary ovarian insufficiency and decreased stature," and discussed in detail the possible endocrine relationships. They state "this syndrome is characterized by infantile sexual organs, complete lack of breast development, small to moderate amounts of axillary and pubic hair in spite of complete absence of other so-called 'secondary sex characteristics,' short stature, the frequency of congenital anomalies, especially webbing of the neck and coarctation of the aorta, late union of the epiphyses, often accompanied by epiphysitis, osteoporosis, precocious senility, an excess of follicle-stimulating hormone in the urine, and a decrease but not an absence of the excretion of the 17-ketosteroids in the urine. The condition is to be differentiated from pituitary dwarfism (pan-hypopituitarism) by the following criteria: *A*. The individuals are short rather than dwarfs. *B*. The bone ages are only slightly retarded rather than markedly so. *C*. These patients have a reduced amount of axillary and pubic hair rather than none at all. *D*. Estrin therapy in these individuals leads to marked increase in axillary and pubic hair whereas it has no such effect in panhypopituitarism. *E*. These patients are quite strong and well-nourished rather than weak and undernourished. *F*. The follicle-stimulating hormone in the urine is increased above the normal rather than absent. *G*. The 17-ketosteroids in the urine are decreased but not minimal. *H*. In an insulin tolerance test these patients exhibit normal hypoglycemia responsiveness as opposed to hypoglycemia unresponsiveness. I. These patients are prone to have other congenital anomalies."

In table 2 are shown various clinical data concerning the cases reported by Turner, by Varney, Kenyon and Koch, by Albright et al., and by Schneider and McCullagh (28), together with five cases that we have observed.[2] In all of the American cases except those of Turner the fact that the ovarian deficiency is not secondary to pituitary failure has been established by the finding of a high titer of urinary gonadotropin[3] as well as the other criteria discussed by Albright. However, it must be admitted that without surgical exploration and histological study it cannot be assumed that all of these cases were instances of complete agenesis of the ovary with absence of germ cells and

[2] *Cases 1, 2* and *3* are reported in detail and are included in table 1. *Case 5* has been biopsied since this paper was written and showed typical agenesis. *Case 4* has not been explored. The cases of Ullrich (29) and Funke (30) have been added from the German clinical literature and doubtless belong to this group. In addition there is a recent report in the Russian literature (31) which describes 6 patients with stunted growth, sexual infantilism, webbed neck and high palate.

[3] It is unfortunate that each of the authors has used a different method of assaying the urinary gonadotropins so that the findings cannot be compared.

TABLE 2

Author		Age	Height inches	Bone Age years	Size at birth	Onset of stunting	Breast	Menstr.	Sexual Hair Pubic	Sexual Hair Axillary	Urinary Gonadotropin	17-keto-steroids	B.P.	Coarct. Aorta	Osteoporosis	Webbed Neck	Eye Abnormalities	Other Abnormalities
Turner (27)	1	16	53	<15		"Always short"	0	0	+							+	Internal squint	
	2	18	55	<15		"Always short"	0	0	±	±					Spine	+		
	3	15¼	50	"Normal"	"Full term"	9 years	0		±	0						+	Internal squint	
	4	16	53.7	<15		"Always short"			0 Labial	0 +					Carpals & metacarpals	Not webbed Short & broad		
	5	23	52.2	<15		8 years			0	+					Generalized Cysts-ulnae	+ Spina bifida		
	6	15	51.7	"Hips Normal"	"Full term"				0	0					Carpal bones	+		Absent R. radial Epiph. Abnormal L. ulnar Epiph.
	7	16	48.5	"Normal"	2½ lb. twin	"Always short"									Slight generalized	+		
Varney & Kenyon (1)	1	26	55.0	14–15	4½ lbs.	"Always short"	0	0	±	±	250–350 M.U./day	2–5 i.u. Capon			Generalized	0	Tubular vision	
	2	19	57.5	14	4½ lbs.	5–6 years	0	0	±	0	250–450 M.U./day	2–5 i.u. Capon			Generalized	0		
Albright et al (2)	1	21	56.5	15–19	"Prematture"	10 years	0	0	+	±	40 M.U./100 cc.	4.7–3.9	138/95			0		
	2	17	54.0	11–13	6 lbs.	"Always short"	0	0	±	±	10 M.U./100 cc.	3.9–3.6	104/70			0		
	3	22	54.0	14–15	"Prematture"	8 years	0	0	0	0	20 M.U./100 cc.	4.7–4.7	120/70		Epiphysitis spine	0		
	4	29	54.0	Complete fusion		"Always short"	0	0	±	±	10 M.U./100 cc.	2.0–3.2			Spine	0		
	5	21	55.5	15	4 lbs.	"Always short"	0	0	±	0	10 M.U./100 cc.	1.0–3.3	150/110	No		0		
	6	28	55.0	Complete fusion	"Full term"	"Stocky"	0	0	±	±	10 M.U./100 cc.	5.1–4.5	134/86			+		
	7	14	53.0	12–13		8 years	0	0	±	0	10 M.U./100 cc.	4.8–5.1	146/88	Yes		0		
	8	18½	55.5	13–15	4 lbs.	10½ years Mastoiditis	0	0	+	+	10 M.U./100 cc.	4.8–5.1	128/80			0		
	9	20	57.5	16–18		Always short	0	0	++ treated	++	>10<30 M.U. /100 cc.	4.3–3.0	140/90	No	Generalized epiph. spine	0	External squint	
	10	37	56.2	Complete fusion		Always short	0	0	±	0	50 M.U./100 cc.	4.9–6.6	134/90		Generalized	0	Bilateral cataracts	Progeria. Heberden's nodes.
	11	30	58.5			12 years	0	0	±	±	10 M.U./100 cc.	0.6–2.7	136/90	No		0		Diabetes; progeria; obesity.

TABLE 2—*Continued*

Author		Age	Height inches	Bone Age years	Size at birth	Onset of stunting	Breast	Menstr.	Sexual Hair Pubic	Sexual Hair Axillary	Urinary Gonadotropin	17-keto-steroids	B.P.	Coarct. Aorta	Osteoporosis	Webbed Neck	Eye Abnormalities	Other Abnormalities
Schneider & McCullagh (28)	1	39	54.0				0	0	±	±	>Normal	2.1				+	0	Cubitus valgus. Mental deficiency.
	2	22	62.0	15–18			0	0	±	±	>Normal	1.8				Short neck	Lack of ret. pigment	Asymmetry of gums
	3	21	54.0	16–17		Time of puberty			±	+		2.4				+		Mental retardation. Deafness.
	4	22	54.0	18		Time of puberty	0		±	±	>Normal	4.2				+		
	5	18	55.0	16			0	0	±	±	>Normal	1.3				Short neck		
Ullrich (29)	1	8	About 45.3		6¼ lbs.	<8 years	Hypoplasia nipples	0	0	0	0	0	0	No	0	+	Bilateral ptosis	Cubitus valgus. High palate.
Funke (30)	1	15¾	54		6¼ lbs.		0	0	0	0	0	0	0	No	0	+	Bilateral ptosis	High palate.
Wilkins & Fleischmann	1	16	51.7	13–14	8½ lbs.	Always short	0	0	±	±	<6 R.U./day [1](14 R.U./day)	2.8 mg. (6.1 mg.[1])	145/95	No	Generalized	0		Mental retardation. Calcified plaques in skin.
	2	17	50.7	15–16	5 lbs.	Always short	0	0	++	+	85 R.U./day	2.2 mg. (5.3 mg.[1])	148/112	Yes	Generalized & Cysts	+ Spina Bifida	Bilateral ptosis	Mental retardation.
	3	16	53.5	14–15	6 lbs.	Always short	0	0	±	0	78 M.U./day	3.1–4.5 mg.	130/100	No	Generalized	0	Myopic Ast. Sl. Exoph.	Underdeveloped jaw. Congenital deafness—one ear.
	4	15	52.8	13–14	8 lbs.	10 years	0	0	+	0			120/70	No	Moderate Cyst-tibia	0		Cubitus valgus.
	5	22	53.0	18–19	4 lbs.	Always short	0	0	++	±	192 M.U./day	3.0–3.6	110/78	No	Moderate	0	0	Cubitus valgus; groove in palate; asymmetry.

[1] After withdrawal of estradiol treatment.

lack of proliferation of the genital epithelium. It is possible that in some instances the development of the ovary had progressed farther and had been arrested at a later stage of fetal life, or during infancy, while in other cases some destructive lesion may have occurred. We have pointed out that in the dwarfed patient autopsied by Tronci (21), there was evidence of proliferation of germinal epithelium.

Discussion Concerning the Cause of the Symptoms

Sexual Abnormalities. Albright explains the sexual abnormalities occurring in these cases by the absence of estrogen and the presence of androgens derived from the adrenal. It is probable that the development of the Müllerian duct system and the suppression of the Wolffian duct development progresses up to a point in late fetal life or early infancy entirely under genetic influence independent of the hormones produced by the ovary, although the possible influence of maternal hormones cannot be excluded. The further development of the tubes, uterus, vagina, nipples and mammae cannot occur without the female sex hormones. When estrins are administered to these patients development then proceeds as in adolescence. Albright contends that the growth of pubic and axillary hair in the female depends primarily upon the adrenal androgens rather than the female hormones. He points out that in "panhypopituitary" dwarfs who uniformly have a very low excretion of the 17-ketosteroids, pubic hair does not develop. In some cases of Addison's disease menstruation may occur but pubic hair fails to grow. Axillary and pubic hair are not lost after normal or artificial menopause. We agree entirely with Albright's hypothesis and have had the opportunity to see it substantiated in the treatment of a hypopituitary female dwarf. At the age of 22 years this girl had no pubic or axillary hair and her breasts, vagina and uterus were infantile. Under treatment with 1 mg. stilbestrol and 25 mg. methyl testosterone daily, a marked growth of pubic hair occurred and the breasts, vagina and uterus developed normally. No enlargement of the clitoris or other evidence of virilization occurred. When methyl testosterone was stopped and stilbestrol alone was given all pubic hair disappeared. The resumption of treatment with 10 mg. methyl testosterone daily caused the reappearance of pubic hair.

It should be pointed out that in the case reports we have collected there is considerable variation in the data regarding abundance of the sexual hair and that in three patients it was completely lacking (Rössle, Turner's *Case 6*, Albright's *Case 3*). In most cases the growth was scanty; in some it occurred only on the labia. In some cases, such as our first, hair was quite abundant on both the pubis and in the axillae. The abundance of hair cannot be correlated directly with the amount of 17-ketosteroids excreted in the urine. Albright has noted that the growth of hair was increased by the administration of estrin. We have observed the same phenomenon in the treatment of our cases. Albright felt that estrogenic therapy through pituitary stimulation increased the adrenal activity. However, it seems equally plausible that estrins and androgens may have a direct synergistic action upon the growth of sexual hair.

Skeletal Growth and Development. Of the eight patients in the foreign literature reported to have merely a primitive genital ridge, all were less than 59 inches tall, except the patient of Meyer (20) who was 66.7 inches tall. The 30 American patients varied from 48.5 to 58.5 inches except for one patient of Schneider and McCullagh (28) who measured 62 inches. In a number of cases the span was relatively long in relation to the height. On the other hand, instead of the long legs which occur in so-called "eunuchoid" individuals, the lower extremities were relatively short in relation to height in our *Case 1*. Unfortunately, complete anthropometric data are lacking on most cases.

As Albright pointed out, the osseous development is much less retarded in these patients than in pituitary dwarfs. The ossification of the cartilaginous centers is usually well advanced, having progressed to the stage just before the fusion of the epiphyses occurs. In most of the cases at least some of the epiphysial fusions have already occurred and in some of the cases all the epiphyses have united with the shafts.

Albright has discussed the possible causes of stunted growth in this condition. We agree

with him and with Varney and Kenyon that it is unlikely the retardation of growth is due to the absence of the ovarian hormones. Likewise there is no evidence that the ovarian deficiency leads to secondary changes in the pituitary resulting in decrease of the growth hormone. Castration in both males and females causes increase in the pituitary gonadotropic hormones without leading to diminution in growth. Albright offers the hypothesis that the impairment of growth is due to the fact that the production of adrenal androgens is less than that of normal adult females. It is quite probable that the growth spurt which occurs during adolescence in girls is brought about by the increase in the adrenal androgens at this time. If the stunting of growth were due to diminished adrenal activity in these patients with ovarian agenesis, one would expect them to grow normally throughout the preadolescent period but fail to make an adolescent growth spurt. The case histories do not indicate that this is so, although detailed data concerning their growth throughout childhood is lacking. In at least 8 cases the history states that the patient weighed 5 lbs. or less at birth and 14 of the patients were said to have been short throughout childhood. In 8 cases the parents stated that the patient's growth had slowed up or ceased at an age between 8 and 12 years. Such a statement must be discounted due to the fact that a moderate retardation in the rate of growth may not attract a parent's attention until this age. Among the various cases collected there is no correlation between the height of these patients and the amount of 17-ketosteroids excreted or the abundance of pubic and axillary hair. Albright's patient *11* was the tallest in his group but had the lowest urinary 17-ketosteroids. Schneider and McCullagh's patient *2* was the tallest among the 30 recorded American patients and had a 17-ketosteroid excretion of only 1.8 mg. per day. Our patient *1* had a relatively abundant growth of pubic and axillary hair but was unusually short. Actually the amount of urinary 17-ketosteroids excreted by these patients is not appreciably lower than that found by Talbot et al. (32) in normal girls between the ages of 12 and 14 years, the period when the growth spurt is at its height. These authors consider amounts of from 2 to 7 mg. as normal for this age group.

It seems more plausible to explain the diminution in growth on a genetic rather than an endocrine basis. The races of pigmies and the so-called "primordial dwarf" of the Tom Thumb type are probably other instances of genetic dwarfism since such individuals present no evidence of pituitary or other endocrine disturbance unless one assumes that there is a selective deficiency of a hypothetical growth hormone. We have pointed out already that the failure of the ovary to develop can be explained only by a defect in the germ. It is well recognized that multiple germinal defects frequently occur in the same individual. It seems probable that ovarian agenesis and stunted growth are merely two defects that are frequently associated and that other congenital abnormalities such as webbed neck, coarctation of the aorta and defects of the eye occur not infrequently. That the association of ovarian agenesis and stunted growth is not invariable is suggested by the patient of Meyer who had ovarian agenesis but was of normal height.

Osteoporosis. In 11 of 30 cases recorded, marked generalized osteoporosis was noted. In three cases there were cystic areas in bones and in five cases Albright noted atrophic changes in the vertebrae. The osteoporosis is probably due directly to ovarian deficiency, as Albright pointed out, and is similar to that seen in the post-menopausal state. It should be noted that in Cushing's syndrome, in which osteoporosis is a prominent symptom, there is also persistence of androgens and diminution of estrogens as in the cases of ovarian agenesis.

Hypertension. The frequent association of hypertension with ovarian agenesis has not previously been emphasized. In only 15 of the cases collected is the blood pressure specifically mentioned. Of these, in 10 instances the systolic pressure was between 130 and 150 mm. and the diastolic pressure between 90 and 112 mm. Taking into consideration that these patients had the stature of children rather than of adults, this may be considered a fairly marked degree of hypertension. In one case of Albright's and in our second case, the hypertension was explained by the presence of coarctation of the aorta. This suggested the possi-

bility that in our other case there might be some other vascular abnormality but none could be demonstrated. We have no explanation for the frequent occurrence of hypertension in this syndrome but wonder whether there can be any relation between the hypertension of ovarian agenesis, Cushing's syndrome and the post-menopausal state since in all these conditions estrogens are decreased and androgens persist.

Other Congenital Abnormalities. Of the 32 cases, webbed neck occurred in 13 and a short neck in 3. An associated cervical spina bifida was found in only two of these patients. In our patient with webbed neck there were abnormalities and fusion of the cervical vertebrae in addition to the spina bifida. Ocular abnormalities occurred in 10 cases: squint, in 3 patients; bilateral ptosis in 3; bilateral cataracts, "tubular vision," mild exophthalmos and lack of retinal pigment each occurred in one patient. Congenital deafness was recorded for two patients and mental retardation in four. These and other abnormalities reported are listed in table 2.

SUMMARY

Three cases are reported of sexual infantilism with stunted growth in which it was demonstrated that the "ovary" consisted of a primitive genital ridge composed only of stroma-like cells and showing no proliferation of epithelium or primordial germ cells. Eight similar cases, studied pathologically, are quoted from the literature. In one of the latter the patient was tall instead of short. Four other cases described in the literature appear to be instances of "arrested development" of the ovary rather than true "agenesis." Three of these patients were over 5 feet tall.

It is suggested that ovarian agenesis is due to a defect of germ plasm causing failure of primordial germ cells to develop or persist in the genital ridge. This in turn leads to a failure in development of the ovaries. However, the defect does not involve the factors of the chromosomes which determine the sex differentiation of the Müllerian and Wolffian ducts and other sexual characteristics. The bearing of these findings on the various theories of sex determination is discussed.

The clinical literature is reviewed and 32 cases including ours resembling the syndrome of ovarian agenesis are tabulated.

The clinical differentiation between ovarian agenesis and pituitary dwarfism with sexual infantilism is reviewed. It is suggested that the growth disturbance is not due to an endocrine disturbance but depends also upon a germinal defect as do the ovarian agenesis and other congenital abnormalities frequently found. That the association of these defects is not invariable is shown by the fact that Meyer's patient with ovarian agenesis was of normal height.

The high incidence of hypertension and of osteoporosis in this syndrome is pointed out. No explanation is offered for the hypertension. Osteoporosis may depend upon ovarian deficiency.

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Some Effects of Testosterone, Testosterone Propionate, Methyl Testosterone, Stilbestrol, and X-Ray Therapy in a Patient with Cushing's Syndrome.[1]

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SEVERAL FORMS of therapy have been tried in the treatment of patients with Cushing's syndrome, at times with seemingly good effect, at times with no apparent benefit (1). Apart from the surgical removal of tumors and hyperplasias of the pituitary and other endocrine glands, the chief forms of therapy have been irradiation of the pituitary region with x-ray and the administration of androgens and estrogens (1-8). Albright et al. (5, 6) have reported the favorable effect observed in their cases under treatment with testosterone propionate, and Rakoff et al. (7, 8) have reported the improvement they observed in two patients treated with stilbestrol. Sosman (9), and, also, Thompson and Eisenhardt (1) have commented upon the improvement, at times dramatic, which may be seen following x-ray irradiation of the pituitary area.

In the patient reported here comparative studies have been made of the effects of testosterone propionate, stilbestrol, and x-ray therapy, and also of methyl testosterone and testosterone. Observations of the clinical results secured by these measures have been accompanied by studies of the effect of these hormones upon the urinary excretion of nitrogen, creatine, creatinine, and the neutral 17-ketosteroids.

[1] Read before the Twenty-Seventh Annual Meeting of the Association for the Study of Internal Secretions, Chicago, Illinois, June 13, 1944.

CASE HISTORY

The patient is a 15-year-old Italian girl who presented herself at the hospital in December, 1941, complaining of a rapid increase in weight, pain in the back, and amenorrhea for 3 months.

The patient weighed 8 pounds at birth and passed through a normal infancy, but between the ages of 4 and 6 years ate poorly and failed to gain at the normal rate. She continued to be thin and underweight until the age of 12 when menarche appeared. At that time her appetite increased markedly and she began to gain weight rapidly. A summary of her changes in weight and height is given herewith.

	Height	*Weight*
January, 1938	54½″	66 lbs.
January, 1939	56	82
September, 1939	56⅞	106
January, 1940	57⅜	118
June, 1940	57¾	123
September, 1940	58½	118
January, 1941	59	115
June, 1941	59¼	106
September, 1941		125
December, 1941		148

This increase in weight was especially prominent in the regions of her face, neck and hips.

Between the ages of 12 and 14, the menstrual periods were normal, recurring at 28-day intervals and lasting for 3 days. Moderate facial hirsutism and considerable hairiness of her back and legs had been noted since childhood. Two years before she came under observation she noticed a progressive increase in facial hirsutism and the development of highly flushed cheeks. She had had acne since menarche; the condition was accentuated during her periods.

She now noted that acne became worse at regular intervals during her amenorrhea, at the times that her periods would have come normally. In the two years which preceded her admission to the hospital she also experienced several severe frontal headaches associated with nausea, short attacks of stabbing pain under her left breast, and intermittent right lumbar pain.

Physical examination on admission showed a cheerful, obese girl with a round, plethoric, hairy face and moderately severe facial and body acne. Pinkish striae were present over the thighs and buttocks. The hair growth was particularly heavy in front of the ears and in the eyebrows, which met in the midline. The hair of the cheeks, chin and upper lip was downy. There was much light-colored hair on the arms and less on the legs, abdomen and back. A line of hair growth extended from the symphysis pubis to the umbilicus. The thyroid was not enlarged. The blood pressure was 145/80 and the heart was essentially normal. No organs or masses were found on palpation of the abdomen. The clitoris was moderately hypertrophied. It was difficult to palpate the pelvic organs per rectum. In spite of the generalized adiposity, with a fat pad overlying the lower cervical vertebrae, the hands and feet were small. Perimetry showed no abnormality in the visual fields.

Laboratory studies. The red-blood-cell count was 6,150,000; hemoglobin, 15.2 gm.; hematocrit, 47%; sedimentation rate, 15 and 5 mm./hour. The white-blood-cell count was 21,000 on admission and 10,000 on discharge. Polymorphonuclear leucocytes were 90%; lymphocytes, 8%; monocytes, 1%; eosinophils, 1%; smear, normal.

The non-protein nitrogen was 38%; total protein, 8 gm.; albumin, 5.4 gm.; and globulin, 2.6 gm. These remained essentially unchanged during the hospital stay. Cholesterol fell from 320 mg. % to 262 mg. %.

The blood chloride was 104 mEq/1; calcium, 5.3 mEq/1; phosphorus, 1.2 mEq/1; alkaline phosphatase, 1.9 Bodansky units; sodium, 143 mEq/1. In two glucose tolerance tests the responses were normal. During stilbestrol treatment the fasting blood sugar rose from 79 mg. % to as high as 230 mg. % and then fell to 100 mg. % without apparent reason.

X-rays plates showed 1) the thymus to be normal and 2) the skull, normal except for a fine granular appearance of the bone; the sella turcica was normal. 3) The right hand and wrist showed slight delay in ossification. 4) The kidneys and bladder presented no abnormality. 5) An intravenous pyelogram demonstrated fair concentration of the dye in 5 minutes; the renal pelves, ureters and bladder appeared normal and there was no evidence of pelvic or adrenal tumor. The lumbar spine and pelvis appeared somewhat osteoporotic. 6) Antero-posterior and lateral views of the chest showed the lungs to be clear, the heart slightly enlarged and to the left, and no evidence of mediastinal tumor.

METHODS OF STUDY

During the periods of observation the patient was confined to the metabolism ward of the Peter Bent Brigham Hospital, where supervised collection of urine specimens could be obtained. A constant diet of 1000 calories, containing approximately 70 grams of protein was administered. During the first hospital admission there were 11.7 gm. of dietary nitrogen, during the second admission, 9.6 gm.[2]

The urine analyses were made daily and in duplicate on 24-hour urine samples. Creatine and creatinine were determined by Folin's method (10). Nitrogen was determined by the macro-Kjeldahl method. The 17-ketosteroids were determined colorimetrically by the method of Friedgood and Berman (11) after extraction with ether and toluol (12).[2]

RESULTS

Period I: Testosterone propionate. The experimental studies, covering a period of 18 months, were divided into four main parts (see figure). During the first part, after an initial control period of observation of 6 days, the patient was given daily intramuscular injections of 25 mg. testosterone propionate for 17 days.

(a) *Nitrogen excretion.* On a constant nitrogen intake of 11.7 gm. per day, the urinary nitrogen excretion exceeded the intake by 1 to 2 gm. per day. On the fifth day of treatment the urinary nitrogen excretion fell below the intake level and continued to decrease over subsequent days of treatment until a fairly constant positive nitrogen balance of 2 to 3 gm. per day was secured. The excretion of nitrogen increased when the administration of testosterone propionate was discontinued and the patient returned to a negative nitrogen balance within 7 days. Calculated on the basis of urinary-nitrogen excretion, the total nitrogen retained during the period of treatment was 32 gm.

(b) *Creatine excretion.* Creatinuria during the control period averaged about 0.5 gm. per day. The excretion of creatine fell to about 0.1 gm. on the seventh day of treatment and subsequently ceased entirely. Immediately upon cessation of treatment, creatinuria reappeared and increased to a peak of 1.25 gm. per 24 hours on the eleventh day of the hormone withdrawal.

(c) *Creatinine excretion.* Creatinine excretion

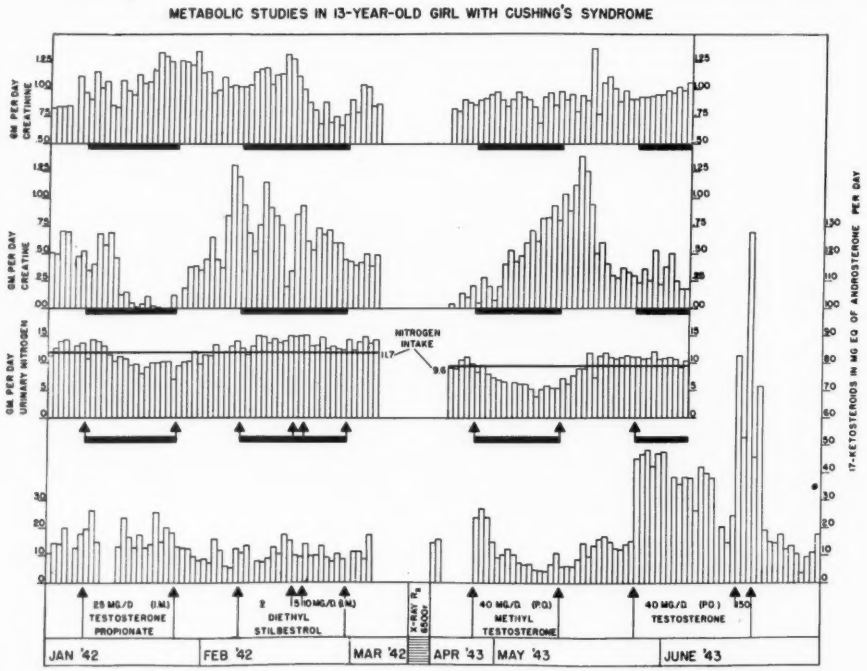
[2] We are indebted to Miss Marion Brian for supervision of the diets and to Miss Mary Sullivan for technical assistance in the determination of the 17-ketosteroids

fluctuated between 0.8 gm. and 1.1 gm. per day during the control period of observation and for the first few days of hormone treatment. Excretion levels of 1.3 gm. were observed during the last week of hormone administration. These elevated levels persisted for approximately a week after the withdrawal of the testosterone propionate, after which the excretion of creatinine decreased to approximately 1.0 gm. per day.

(d) *Neutral 17-ketosteroid excretion.* The urinary 17-ketosteroid excretion ranged from 11 to 20 mg. per 24 hours during the control period of 6 days. The administration of testosterone propionate resulted in irregular increases of 17-ketosteroid excretion (12 to 26 mg. per 24 hours). Withdrawal of the hormone was followed by a gradual decrease in excretion (5 to 15 mg. per day).

(3) *Clinical effects.* The administration of testosterone propionate was discontinued because the patient complained of increasing hirsutism and acne. It was observed that the clitoris became more hypertrophied during testosterone-propionate treatment.

Period II. Diethylstilbestrol. A recovery period of 12 days followed the period of testosterone-propionate administration. The patient was then given daily intramuscular injections of diethylstilbestrol, in successively increased doses of 2, 5, and 10 mg. for 20 days. Measurements of metabolism were continued for 6 days after the administration of stilbestrol was stopped.



(a) *Nitrogen excretion.* On 2 mg. of stilbestrol per day, the nitrogen balance remained negative to the same degree as it had been during the original control period. A tendency toward nitrogen equilibrium developed during the latter half of the stilbestrol treatment with doses of 10 mg. per day.

(b) *Creatine excretion.* The effect of stilbestrol on the excretion of creatine is difficult to judge from our data, partly because the level of creatine excretion had not become stabilized following the withdrawal of testosterone propionate. There appears to be some evidence that successive transient increases in creatinuria appeared each time the dose of stilbestrol was raised from 2 to 5 to 10 mg. per day. In

general, the average level of creatine excretion during stilbestrol administration was somewhat in excess of that of the control period.

(c) *Creatinine excretion.* There was a decrease in the level of creatinine excretion during the latter part of the period of stilbestrol therapy. Withdrawal of stilbestrol was followed by a return to control levels.

(d) *Neutral 17-ketosteroid excretion.* The urinary excretion of 17-ketosteroids during stilbestrol therapy was slightly below that of the control period (10 mg. per day).

(e) *Glucose metabolism.* The fasting blood sugar level rose from 80 to 138 mg. per cent on 2 mg. of stilbestrol per day, and finally to 230 mg. per cent on 10 mg. per day. The level subsequently declined to 100 mg. per cent despite continuance of the 10 mg. dosage.

(f) *Clinical effects.* On stilbestrol therapy the acne of the face and chest decreased significantly, the pronounced facial plethora diminished, and the clitoris became somewhat less hypertrophied. The facial hirsutism continued to increase. The use of stilbestrol was associated with various untoward reactions: nausea, headache, dizziness, blurring of vision, and sensations of being hot and cold. Symptoms appeared 1 hour after injections and lasted 3 to 4 hours.

Roentgen therapy. In view of the fact that there was no striking clinical improvement with stilbestrol and because of the symptoms induced by the medication, the patient was transferred to the x-ray department. Three series of treatments were given, each consisting of 8 daily exposures of the hypophysial region through 2 portals (right and left temporal areas). A total of 2400 r was administered in April, 1942; 2000 r in July, 1942; and 2080 r in February, 1943.

When re-examined in September, 1942, the patient showed definite symptomatic improvement. There was little change in weight but her appetite, which had been ravenous, had decreased to normal proportions. She was more alert mentally and physically; and her skin, particularly that of the cheeks, was less red and tense. She had noted that the hair of her face was not growing so fast as it had previous to x-ray therapy. The hair of her forearms also thinned out temporarily. In October, the patient noticed that her appetite was getting out of control again although she seemed otherwise to have maintained her general clinical improvement. She still complained, however, of pain in her spine and x-ray examination showed osteoporosis of thoracic vertebrae and a peculiar granular appearance of the calvarium similar to that seen in hyperparathyroidism and basophilism. Further clinical improvement was observed after her series of x-ray treatments in February, 1943. On March 25, 1943, a normal menstrual period occurred for the first time since the onset of her amenorrhea.

Period III. Methyl testosterone. The patient was readmitted to the metabolism ward in April, 1943. After an initial 8-day period of study she was given methyl testosterone orally in divided doses totalling 40 mg. per day for 16 days. Her recovery from the effects of this form of testosterone was then observed over a period of 14 days.

(a) *Nitrogen excretion.* On a constant nitrogen intake of 9.6 gm. per day, approximate nitrogen balance was obtained during the control period. Administration of methyl testosterone was followed by an immediate decrease in urinary nitrogen excretion. Over a period of 11 days urinary nitrogen decreased to a minimum of 5 gm. per day. It remained at this level for the final 5 days of treatment. Following cessation of treatment, the nitrogen excretion increased until a slightly negative balance characteristic of the control periods was again evident. A total nitrogen retention of 58 gm. was secured by the 16-day period of methyltestosterone administration.

(b) *Creatine excretion.* Perhaps as a result of the beneficial action of the recent periods of x-ray therapy, creatine excretion on readmission to the metabolism ward in April, 1943, had decreased to about 0.1 gm. per day, in contrast to the admission and discharge levels of 0.5 gm. per day observed during her earlier period of hospital investigation. Administration of methyl testosterone was followed in about 5 days by an unequivocal increase in creatinuria, the levels progressively rising to 0.85 gm. per day during the period of treatment and continuing to rise to a maximum of 1.35 gm., observed on the fourth day after treatment was discontinued. The creatinuria then decreased

over a period of 6 days to a fairly constant level of 0.25 gm. per day.

(c) *Creatinine excretion.* It is doubtful whether methyl testosterone had an appreciable effect on creatinine excretion, levels of the order of 0.80 gm. being obtained throughout the control, therapeutic, and recovery periods.

(d) *17-ketosteroid excretion.* An elevated 17-ketosteroid excretion was observed during the first 3 days of methyl testosterone administration. Whether this could be attributed to the methyl testosterone is not clear. It is certain, however, that there was a subsequent decrease in 17-ketosteroid excretion, which reached 5 mg. per 24 hours during the latter part of this experimental period, and persisted at about this level for several days after the cessation of treatment. It then rose to 10 mg. per day.

Period IV. Testosterone. After a period of recovery from the effects of methyl testosterone, the patient was given 40 mg. of testosterone per day by mouth for 19 days. The dosage was increased to 150 mg. per day for the last three days of observation. Nitrogen, creatine, and creatinine studies were carried out only during the first 10 days of treatment with testosterone. Excretion of 17-ketosteroids was followed during the entire period of therapy.

(a) *Nitrogen excretion.* No significant effect on nitrogen excretion was observed during 10 days of treatment with testosterone.

(b) *Creatine excretion.* The creatine excretion prior to the administration of free testosterone was about 0.25 gm. per 24 hours. During the oral administration of this hormone there were irregular increases ranging between 0.35 and 0.5 gm. per 24 hours.

(c) *Creatinine excretion.* The excretion of creatinine changed but little. A progressive rise from 0.9 to 1.05 gm. per 24 hours was observed.

(d) *The 17-ketosteroid excretion.* With oral administration of 40 mg. of free testosterone, the 17-ketosteroid excretion increased abruptly. On the first day the level of excretion rose from 15 mg. to 45 mg. A level of 38 to 48 mg. excretion was maintained during the 10-day period of hospital observation. The levels observed in urine collections made subsequent to discharge from the hospital were significantly lower in several instances while the patient was reputedly on 40 mg. per day. With the administration of 150 mg. of free testosterone per day for 3 days there was a striking but variable increase in the 17-ketosteroid excretion. The physiological significance of these variations is questionable because the patient was disturbed by the androgenic effects of this hormone and may have made an incomplete collection of urine or neglected to take the full amount of medication. On cessation of testosterone administration the 17-ketosteroid excretion returned abruptly to the pre-treatment level of 15 mg. per day.

DISCUSSION

The administration of androgen or estrogen and the irradiation of the pituitary area with x-ray have produced in our patient results that are in general accord with earlier reports of the effects of these forms of therapy in Cushing's syndrome (1–9). The intramuscular administration of testosterone propionate in 25-mg. doses brought about a positive nitrogen balance of several grams per day (13–21) and caused a marked diminution of creatine excretion (17–20, 22, 23). The oral administration of methyl testosterone in 40 mg. dosage produced a somewhat greater positive nitrogen balance (21, 24–30) and in contrast to testosterone propionate caused an increase, rather than a decrease, in creatinuria (23, 30–32).

With these androgens, less striking and indeed somewhat questionable effects were observed on creatinine excretion and on the excretion of neutral 17-ketosteroids. There was evidence of an increased excretion of 17-ketosteroid during treatment with testosterone propionate and in contrast a decrease during the period of methyl testosterone administration (6).

In marked contrast to the effects secured with the two testosterone derivatives, that is, with testosterone propionate and methyl testosterone, oral administration of free testosterone in doses of 40 mg. per day caused, during the 10-day period of observation, no significant change in the excretion of nitrogen, creatine, or creatinine. On the other hand, daily administration of this quantity of free testosterone caused a marked increase in 17-ketosteroid

excretion; and it was found possible on increasing the dosage of testosterone to 150 mg. per day to obtain levels of 17-ketosteroid excretion in excess of 100 mg. per 24 hours.

On the basis of the above observations it is tempting to speculate upon the effect that methylation and esterification may have upon the mode of action of testosterone and upon its inactivation in the body. It is generally concluded that testosterone is inactivated in the liver with the production of 17-ketosteroids (33–37) and it is usually assumed that parenteral routes of administration enhance the effectiveness of testosterone compounds by permitting them to reach their sites of action without preliminary passage through the liver (26–28). Summary of the effects which these variations in route of administration and in molecular structure have upon the mechanism of testosterone action awaits the accumulation of further data. To this end it would be helpful to know whether the creatinuria produced by the oral administration of methyl testosterone is also observed when the hormone is given parenterally (26–28); and similarly, whether parenteral administration of free testosterone is also productive of a marked increase in 17-ketosteroid excretion.

In our patient the administration of testosterone propionate and of methyl testosterone was not productive of demonstrable clinical improvement despite the definite effect which these hormones were shown to have had upon her nitrogen balance. This fact is rather in contrast to the observations of Albright et al. (5, 6), who found their patients considerably improved by the administration of testosterone propionate. The difference in clinical effect is perhaps explicable on the basis that our patient was in an earlier or less severe phase of the disease than that experienced by Albright's patients. Our patient did not exhibit a great degree of muscle-wasting and in consequence might not be expected to benefit significantly from the moderate increase in muscle protoplasm and nitrogen storage brought about by the administration of androgen. On the basis of later observations Albright et al. (6) have demonstrated that a markedly negative nitrogen balance (38) is not a "sine qua non" of Cushing's syndrome. It becomes doubtful, therefore, whether specific importance can be attached to the negative nitrogen balance and muscle wasting that is observed chiefly in the late stages of this disease.

So far as our patient was concerned, administration of testosterone propionate, methyl testosterone, or free testosterone was productive of no clinical improvement. Continued use of the androgens appeared contra-indicated because of the distressing increase in acne, hirsutism, and virilism that was associated with their administration.

Evaluation of the effects of stilbestrol in the present study is complicated by the fact that the drug was administered before complete recovery from the effects of testosterone propionate had occurred and also by the fact that the drug was given in large and poorly tolerated dosage. Relatively little effect upon nitrogen excretion was observed. There was a tendency to increased creatinuria, which in view of the observations of Knowlton et al. (20) upon creatinuria induced by another estrogen (estradiol benzoate) perhaps deserves comment. Knowlton et al. (20) observed increased creatinuria during estrogen therapy only in their masculinized girl; no effect of estrogen upon creatinuria was observed in their normal women or in their women with hypogonadism. It would be interesting to know whether this effect of estrogen can be consistently obtained in masculinized women, both those with Cushing's syndrome and those showing simple virilism. Soffer et al. (39) and also Albright et al. (40) have called attention to other examples of dependence of hormone action upon the underlying endocrine status of the patient. The former observed a paradoxical increase in sodium-chloride excretion following desoxycorticosterone-acetate administration in patients with Cushing's syndrome, and the latter, a similar paradoxical loss of salt and water in these patients following methyl-testosterone administration.

The decrease in creatinine excretion observed in our patient during stilbestrol therapy deserves consideration. The usual constancy of creatinine excretion in man (41–43) makes one hesitate to accept as consequential a single series of observations showing this effect. Beard et al. (44) have reviewed their theories

of the inter-relationship of creatine and creatinine metabolism, summarizing their view that creatinine may be converted to creatine under conditions of increased tissue hydration. In this regard it is perhaps worth mentioning that in our patient the period of decreased creatinine excretion under stilbestrol therapy was associated with a generally increased creatinuria. However, a similar association was not observed during the period of increased creatinuria induced by methyl testosterone and the theories and conclusions of the Beard school are in disagreement with the observations of Schoenheimer et al. The latter workers deduced from isotope studies that creatine may be converted to creatinine, but that the opposing hydration of creatinine to creatine does not occur (45).

Rakoff et al. (7, 8) treated two female patients with Cushing's syndrome with stilbestrol and observed a decrease in hirsutism and in size of the clitoris. In one of these patients stilbestrol appeared to cause a pronounced accentuation of the original diabetes. In our patient stilbestrol caused an improvement in the acne and a decrease in the size of the clitoris. The improvement was not striking and in the dosage used the drug produced a number of mild toxic reactions.

The fasting blood sugar was observed to rise during the period of stilbestrol administration and reached 230 mg. per cent on the 10 mg. dosage. The effect upon the blood sugar was temporary and in a few days the fasting blood sugar level returned to normal despite continuance of stilbestrol medication. Ingle (46) has observed that permanent diabetes may be produced in partially pancreatectomized rats and that a transient period of diabetes may be induced in normal rats by stilbestrol administration. The fact that the hyperglycemia induced in our patient was transient appears in conformity with the fact that her blood sugar tolerance curves and fasting blood sugar levels in the control periods were essentially normal.

Irradiation of the pituitary region with x-ray was followed in our patient by a gratifying remission of the disease. The ravenous appetite disappeared and there was improvement in acne and hirsutism. Normal menstrual periods made their appearance in March, 1943, following the third series of x-ray treatments and have persisted to date. There has been little change in weight, but the skin, particularly that of the cheeks, has become less red and tense. Whether these signs of improvement are temporary or permanent and whether they may be sustained if necessary by further x-ray treatment is a matter of hazard. Spontaneous improvement has occurred in other patients (29) and there are several instances of both temporary and permanent improvement (1, 9) following x-ray therapy.

The rationale for applying x-ray therapy to the pituitary region in Cushing's syndrome seems clear. The primary pathological changes in the disease are found in the basophile cells of the anterior pituitary lobe. As described by Crooke (47), these changes are interpreted as either hyperfunctional (47) or as the end result of hyperfunction (48). The frequently associated tumors of the pituitary, adrenal, and other endocrine glands (1) are thought to be of secondary etiological importance. They should be searched for and removed when present, but in their absence, the possibility of affecting the hyperfunction of the basophile cells by irradiation with x-ray is worthy of careful consideration and trial.

SUMMARY

The metabolic and clinical effects of testosterone propionate, stilbestrol, methyl testosterone and testosterone were observed under controlled conditions in a girl, aged 15, with Cushing's syndrome. Injections of testosterone propionate, 25 mg. per day, caused a diminution in nitrogen excretion and a disappearance of creatinuria. Stilbestrol, 2 to 10 mg. per day, intramuscularly, did not affect nitrogen excretion but perhaps induced a moderate increase in creatine excretion and a decrease in creatinine excretion. Methyl testosterone, 40 mg. per day, orally, caused a striking decrease in nitrogen excretion and a tremendous increase in creatine excretion. Creatinine excretion remained unaltered. Testosterone, 40 mg. per day, orally, did not produce unequivocal changes in excretion of nitrogen, creatine or creatinine.

Irregular increases in 17-ketosteroid excretion, with levels as high as 26 mg. per day, oc-

curred during administration of testosterone propionate. During recovery from this hormone and also in the period of stilbestrol administration, the 17-ketosteroid excretion averaged about 12 mg. per day. Methyl testosterone caused an initial 3-day increase in 17-ketosteroids from 15 up to 25 mg., followed by a depression to levels of about 5 mg. Free testosterone caused a precipitous and marked rise in 17-ketosteroid excretion. Thirty-eight to 48 mg. of ketosteroid was excreted on the 40-mg. dose and over 100 mg. on the 150-mg. dose. The pattern of biochemical activity of these hormones suggests marked differences in their metabolism.

The androgens induced distressing accentuation of acne, hirsutism and hypertrophy of the clitoris. Stilbestrol improved these conditions but the benefit was not striking and was associated with certain untoward reactions and a transient hyperglycemia. X-ray therapy appeared to improve the subjective and objective manifestations of the disease.

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Further Studies of the Absorption, Distribution, and Elimination of Thiouracil[1]

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IT IS NOW well established (1–8) that thiouracil is very effective in controlling thyrotoxicosis. However, it has been shown to have toxic effects which interfere with freedom in its use. It is possible that the frequency of complications from this drug can be appreciably reduced when more is known about its metabolism.

The establishment of the minimal effective dosage in the treatment of thyrotoxicosis must be made clinically, but this may be hastened by biochemical studies of thiouracil. Although many estimations have been made of the concentration of thiouracil in the thyroid glands of thyrotoxic patients (9), the minimal quantity of the drug necessary to cause adequate inhibition of thyroxin production has not been determined. In ascertaining this point it seems reasonable that the dosage of thiouracil should be adequate to keep its concentration in the blood at a fairly even level.

In a previous report (10) we have described some of our studies dealing with the absorption, distribution and excretion of thiouracil. We have investigated further the pharmacology of thiouracil and are presenting the results in this report.

METHODS

Most of the studies were conducted in man, but some were made on rats.

All thiouracil[2] given orally was in the form of 0.1 gm. tablets, while that given intravenously was in the form of a 1 per cent solution of its sodium salt in physiological saline, the pH of which was adjusted to about 8.5. Urine specimens were preserved with chloroform. All of the analyses for thiouracil were according to the methods of Williams, Jandorf and Kay (11).

In the analysis of the content of thiouracil in rats, the animals were killed by a blow on the head and were ground up immediately in a meat grinder. The tissue was then mixed with sodium hydroxide and trypsin in a Waring Blendor and the analysis continued as previously described (11).[3]

In the observations of patients, estimations of the fractions of sulfur in the urine were made by the gravimetric method of Folin and Benedict (12). The total sulfur in the stools was determined by the method of Benedict (12) while the inorganic and ethereal fractions were estimated according to the titrimetric method of Fiske (12).

Other analyses were conducted by the following methods: thiocyanate, using ferric nitrate (13); thiosulphate, using potassium iodate (14); cystine, by Sullivan's test (15); cysteine, by Sullivan's test (15); uracil, by Johnson's method (16); melanin, using bromine water (17); and urochrome, by the Weis test (18).

[1] Aided in part by a grant from the Milton Fund of Harvard University.

[2] Thiouracil in this investigation was supplied through the generosity of Lederle Laboratories, Inc., under the trade name "Deracil."

[3] In the estimation of the thiouracil content of blood, immediately preceding precipitation it is advisable to adjust the pH to approximately 7. When the solution is distinctly alkaline sufficient copper ions may appear in the filtrate to give a blue color which will interfere with colorimetric estimations of thiouracil.

RESULTS

Absorption of Thiouracil from the Gastro-Intestinal Tract

Thiouracil is rapidly absorbed from the gastro-intestinal tract. For example, when 0.1 gm. was given to a fasting individual, the maximal level in the blood was reached in 30 minutes (fig. 1). It has been shown (10) that all of the drug is absorbed or is broken down in the gastro-intestinal tract; none of it appears in the stools. More than 80 per cent usually disappears from the gastro-intestinal tract within 2 hours. An attempt was made to gain an index of the relative proportions of thiouracil absorbed and the quantity destroyed by comparing the amount of thiouracil excreted in the urine following the intravenous injection of the drug with that following the ingestion of an equivalent amount by the same individual a few days later. In attributing the difference in the quantity excreted under these circumstances to the approximate amount of thiouracil destroyed in the gastro-intestinal tract, the assumption is made that all other factors remained reasonably constant. In 2 normal subjects 0.1 gm. was used as a test dose while in a third 0.2 gm. was used. In each individual the total excretion of thiouracil was greater (fig. 2), averaging about 15 per cent more, after it was given intravenously than when given by mouth since it gains entrance to the blood stream immediately and in high concentration. It was found that when thiouracil was given orally it did not appear in the urine in the first 30 minutes and its excretion was complete in 24 hours. On the other hand, when it was given intravenously, although appreciable quantities were excreted in the first 30 minutes the drug continued to be eliminated during the second 24-hour period. The query may be raised as to whether this apparent paradox was due to the fact that when the circulating blood cells were exposed to a high concentration of thiouracil they acquired appreciable quantities of this drug and only slowly liberated it. It has been shown *in vitro* that thiouracil is not readily liberated from blood cells (10).

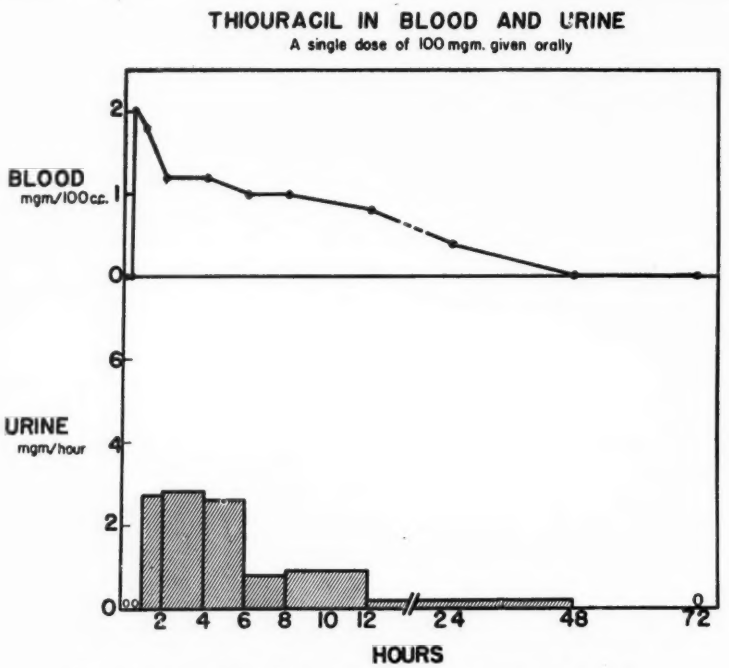

FIG. 1. Amount of thiouracil in blood and urine following the ingestion of 0.1 gm. of thiouracil by a normal individual.

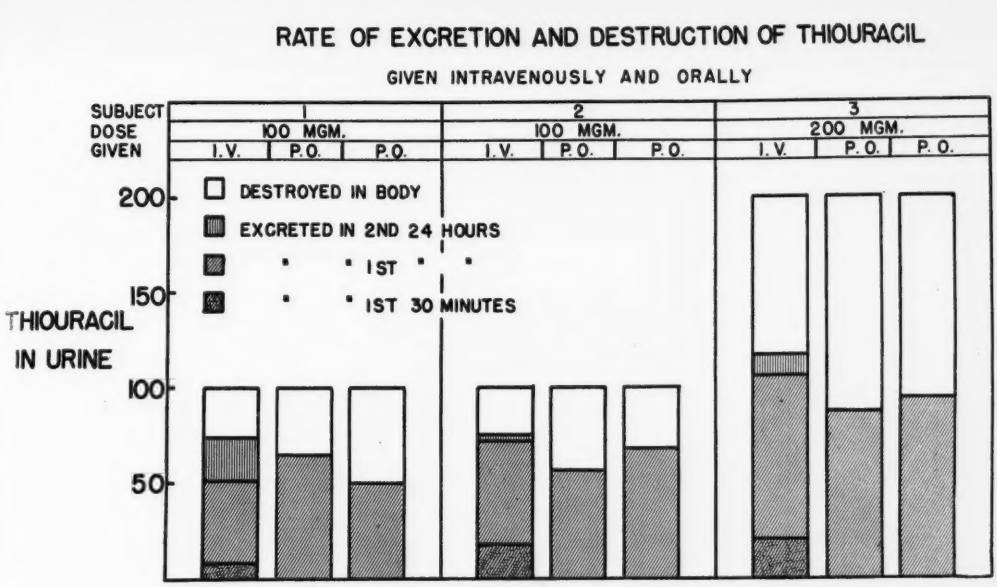

FIG. 2. Note that in each of the 3 individuals tested the quantity of thiouracil excreted in the urine was greater when it was injected intravenously (I.V.) than when the same quantity was given by mouth (P.O.). With the intravenous administration of the drug its excretion began earlier and lasted longer than when it was ingested. The quantity of the drug not recovered (blank column) is an indication of the amount destroyed in the body, including the gastro-intestinal tract.

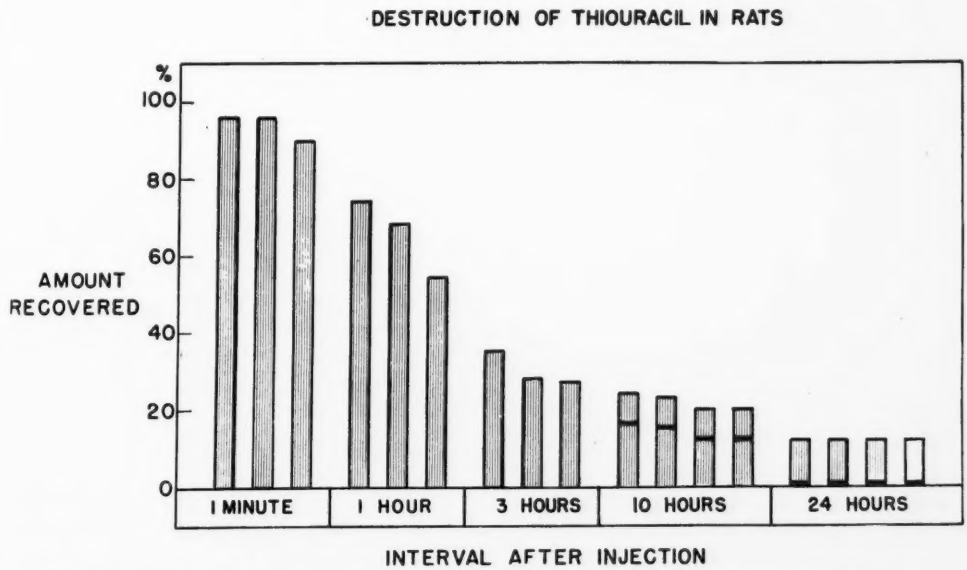

FIG. 3. The quantity of thiouracil recovered from the entire rat carcass one minute after its intravenous injection indicates the accuracy of the method. The amount of the drug not recovered represents roughly the quantity destroyed in the body. The portion of the columns above the broad bars indicates the total amount of thiouracil excreted in the urine. In other instances the urine passed during the experiment was added to the carcass and only a single determination performed. Note the very rapid rate of destruction in the first 3 hours; only traces were present after 24 hours.

Destruction of Thiouracil in the Body

It is assumed that essentially all of the drug not appearing in the urine was broken down in the body or in the gastro-intestinal tract. The proportion varies in accordance with the size and frequency of dosage, the amount of intake

In the experiment illustrated in figure 2 it is observed that when one subtracts the quantity of thiouracil excreted within 30 minutes after its injection from the total excretion, the result is approximately equal to the total amount excreted after the drug is given orally. This

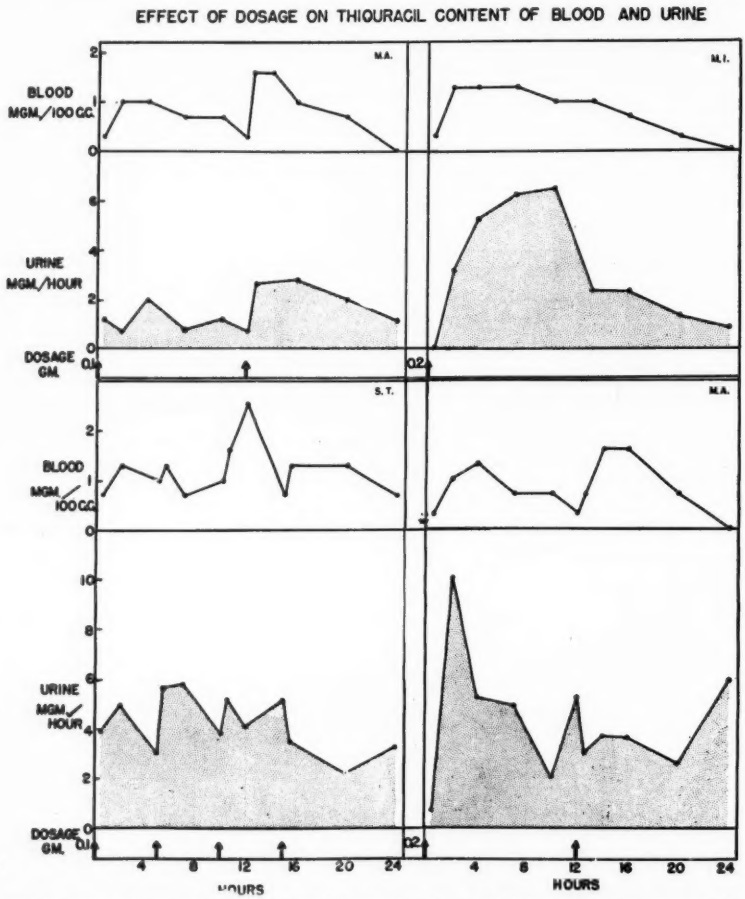

FIG. 4. Two subjects were normal and one (S.T.) had thyrotoxicosis. Each had received the dosage of thiouracil indicated by the arrows for 5 days or longer. The time of administration of the drug conforms closely to that we have routinely employed in the treatment of many thyrotoxic patients. In each instance the initial daily dose was given at 7 a.m. (zero hour in figure) and none was given during the sleeping hours. Changes in the quantity of thiouracil in the blood and urine can be observed soon after each dose. Note the relatively small concentration of the drug in the 3 a.m. (20-hour) and 7 a.m. (24-hour) specimens.

of fluids, the rate of absorption from the gastro-intestinal tract, the kidney function, and perhaps other factors. In many thyrotoxic patients receiving total daily dosages of from 0.4 to 1.0 gm. of thiouracil it has been found (10) that about one-third of this quantity is excreted in the urine.

suggests that the rate of destruction of thiouracil in the body is similar whether it is administered intravenously or orally. The quantity of the drug destroyed in the gastro-intestinal tract, when it is ingested, is roughly equal to the amount excreted in the urine within 30 minutes after its injection intravenously.

In order to ascertain the rate and amount of destruction of thiouracil taking place in the body under standard conditions, 5 mg. of this drug was injected into the femoral vein of each of 17 rats, each weighing about 125 gm. The animals were not given food for 12 hours before or during the test, but they were permitted to have water until the experiment began. as described under "Methods." The 1-minute experiment served to test the accuracy of the method; an average of 94 per cent of the injected thiouracil was recovered (fig. 3). At the end of 1 hour an average of 65 per cent was recovered; at 3 hours, 30 per cent; and after 10 hours, 22 per cent, of which 8 per cent was found in the urine. After 24 hours no more than

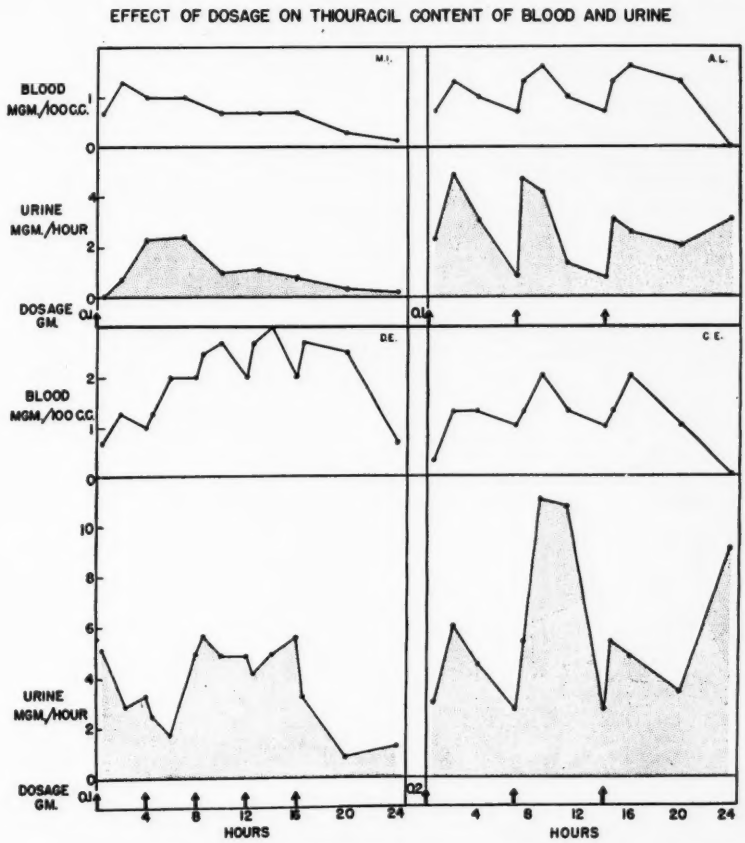

FIG. 5. Two subjects were normal and 2 (D.E. and A.L.) were thyrotoxic. See legend for figure 4.

They were killed at intervals of 1 minute and 1, 3, 10, and 24 hours thereafter. Those killed after 10 and 24 hours had been kept in metabolism cages and the urine was collected in tubes containing chloroform. Any urine passed by the other rats during the experiment was added to the carcass. The entire animal was ground and analyzed for the thiouracil content a trace of thiouracil was found in the carcass and only 12 per cent had been excreted in the urine. The very rapid destruction taking place in the first 3 hours and the much slower changes in the subsequent 7 hours suggest that destruction is very rapid when the concentration of thiouracil in the body is high but much less rapid when the content is low.

Effect of Dosage on the Thiouracil Content of the Blood

In the treatment of thyrotoxic patients dosages varying from 0.1 to 0.6 gm. daily are usually employed (8). A single dose ordinarily is 0.1 gm. although frequently it is 0.2 gm. In figures 4 and 5 are shown the variations in the concentration of thiouracil of the blood with different systems of dosage.

When 0.1 gm. of the drug is given twice daily, its concentration in the blood tends to be maintained at a higher level than when the same quantity is given in one dose, and, similarly, the concentration is greater when 0.1 gm. is given 4 times daily than when 0.2 gm. is given twice. In general, the individuals receiving small doses frequently were found to maintain a greater concentration than those receiving larger doses less frequently, but with all of the systems of dosage there was observed a distinct lability in the blood level of thiouracil throughout the day. In none of the patients was the drug given during the sleeping hours and the concentration in the blood tended to be low the following morning. On the discontinuation of therapy no thiouracil was demonstrable in the blood after intervals varying from a few hours to 3 days, when the total daily dosage had been less than 0.6 gm. With larger dosages it has been found in the blood for 5 days after cessation of treatment although in all instances the quantity present became small within one day after discontinuation of therapy.

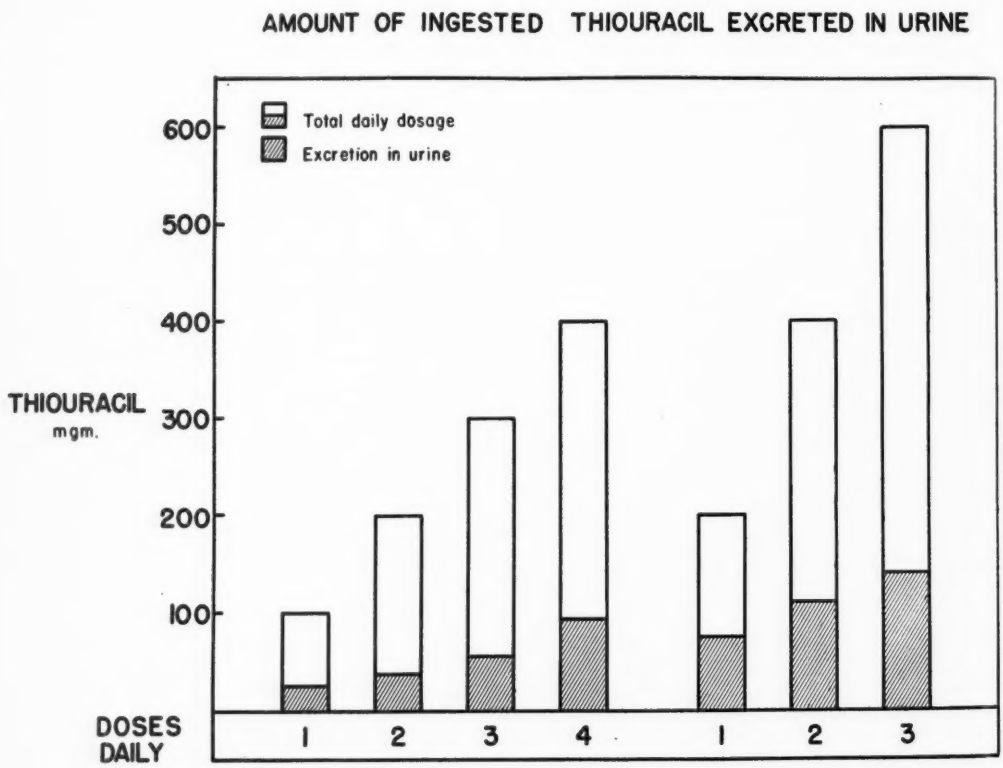

FIG. 6. In each of 7 individuals the total quantity of thiouracil excreted in the urine per 24 hours was determined. The dosage indicated had been given for several days preceding the collection of urine. Note the small proportion of thiouracil ingested that was excreted in the urine. Doses of 0.2 gm. given once or twice daily caused a greater excretion of the drug than when one-half this dose was given twice as often. The larger the total daily dosage the smaller was the percentage of thiouracil excreted.

Distribution of Thiouracil in the Tissues and Body Fluids

Thiouracil has been found (10) in essentially all of the fluids and tissues of the body of patients; the pituitary, thyroid, adrenals, bone marrow and milk show the greatest concentrations. There have been no studies reported of the thiouracil content of fetuses. Four pregnant rats were given 0.1 per cent thiouracil in water as the only source of liquid during the last 2 weeks of pregnancy. At term Cesarian sections were performed and estimations were made of the concentration of thiouracil in the mothers, placentae and fetuses. When expressed in terms of dry weight the concentration in the mothers was roughly twice that in the fetuses, but the concentration in the placentae tended to be slightly greater than in the mothers. That the drug was active in the fetuses was shown by the fact that they weighed approximately 20 per cent less than the control rats, goiters were present and there was marked hyperplasia of the acinar cells of their thyroid glands.

Effect of Dosage on the Excretion of Thiouracil in the Urine

Within an hour or two after the ingestion of thiouracil by man an appreciable quantity of it was recovered from the urine (figs. 1, 4, 5, 6). It was found that the greater the total daily dosage the smaller was the percentage of it excreted. However, a larger quantity of the drug was excreted when 0.2 gm. was given once or twice daily than when one-half of this dose was given twice as often (fig. 6). Usually no thiouracil was found in the urine within 1 to 3 days after discontinuing therapy when the daily dosage was less than 0.6 gm.

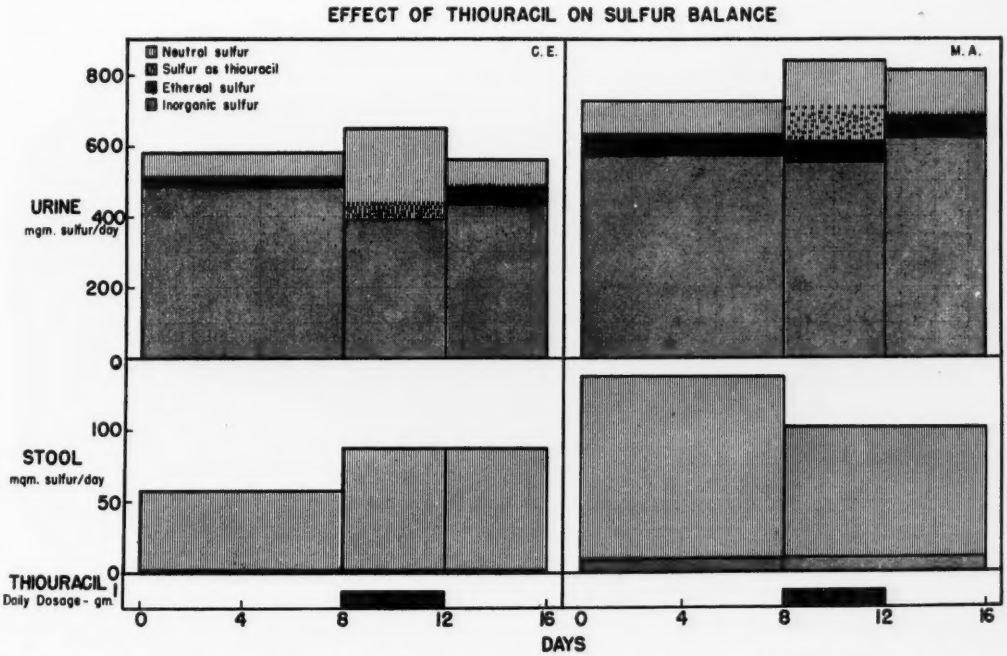

FIG. 7. Two normal individuals were placed on a constant diet, C.E. received 789 mg. of sulfur per day and M.A. 976 mg. After having been maintained on this diet for 5 days the control period was started. Thiouracil was given in doses of 0.2 gm. 5 times daily; thus, 250 mg. of sulfur was supplied daily in this form. Note the increase in neutral sulfur and the decrease in inorganic sulfur in the urine associated with the ingestion of thiouracil.

Effect of the Administration of Thiouracil on the Excretion of Sulfur

Since the amount of thiouracil excreted in the urine of man accounts for only a small proportion of that ingested, and since none of

it is found in the stools, a study was made of possible break-down products by determining the quantity of various fractions of sulfur excreted in the urine and stools after the administration of thiouracil. For this study 2 normal individuals were maintained on a constant diet and estimations were made of the quantity of inorganic, ethereal and neutral fractions of sulfur in the urine and stools before, during and after the ingestion of thiouracil. The amount of thiouracil excreted was also determined. The drug was given in doses of 0.2 gm. 5 times daily for 4 days; this dosage supplied 250 mg. daily

normal individuals who were maintained on a constant diet. Even during the first 4 hours following the injections there was demonstrated (fig. 8) an increase in the excretion of neutral sulfur but a decrease in the inorganic and ethereal fractions. In one individual these changes persisted for several days. The injection of 460 mg. of thiourea, intravenously, into one subject produced changes in the excretion of sulfur similar to those following the injection of thiouracil.

It is not known what substances accounted for the increased output of neutral sulfur frac-

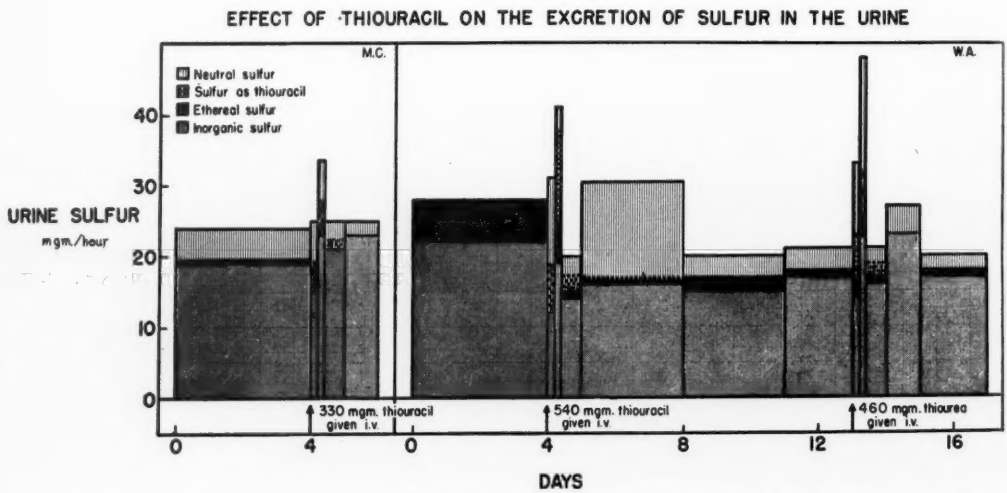

FIG. 8. Two normal individuals were placed on a constant diet. M.C. received 880 mg. of sulfur per day and W.A. receiving 1160 mg. After having been maintained on this diet for 5 days the control period was started. Note the increase in neutral sulfur and decrease in the ethereal and inorganic sulfur occurring even during the 4 hours following the injection of thiouracil intravenously. An increase in neutral sulfur and a decrease in inorganic also promptly followed the injection of thiourea in one subject.

of sulfur. The thiouracil produced an increase in the quantity of neutral sulfur excreted in the urine and a decrease in the inorganic sulfur (fig. 7). Less than one-half of the increased excretion of neutral sulfur was in the form of thiouracil. In one subject no ethereal sulfur was excreted while the drug was being administered, but in the other subject no change in the quantity of ethereal sulfur was observed. In neither individual was there a significant change in the quantity of sulfur excreted in the stools.

The amounts of these sulfur fractions were also estimated in the urine before and after the injection intravenously of thiouracil into 2

tion. Less than half of it was in the form of thiouracil or thiourea. No thiouracil was found to be changed to thiourea (11). None of the break-down products of thiouracil in the urine were demonstrated to be in the form of thiocyanate, thiosulphate, uracil, cysteine, cystine, urochrome or melanin; however, the lack of sensitivity of some of the tests is to be borne in mind.

Following the administration of thiourea to normal individuals, Medes (19) observed an increase in the excretion of neutral sulfur and of cystine. Blood and Lewis (20), however, found no increase in cystine in the urine after the administration of thiourea to rabbits.

SUMMARY

Comparison of intravenous with oral administration of single doses of 0.1 or 0.2 gm. of thiouracil to normal individuals indicate that approximately 15 per cent of the quantity ingested is destroyed in the gastro-intestinal tract.

The percentage of thiouracil excreted in the urine varies in accordance with the size and frequency of dosage as well as with other factors. None of the drug is excreted in the stools, but when it is given daily about one-third of it is excreted in the urine; so it may be assumed that approximately 50 per cent is broken down in the body tissues and fluids.

When 5 mg. of the sodium salt of thiouracil was injected intravenously into rats and the entire carcass analyzed, rapid destruction of the drug was demonstrated. More than 65 per cent was broken down within 3 hours and only traces were found after 24 hours.

Sulfur balance studies in 4 normal subjects showed that the administration of thiouracil caused an increased excretion of neutral sulfur, a decrease in inorganic sulfur and usually a decrease in ethereal sulfur. Thiouracil partially accounted for the increased excretion of neutral sulfur; the remainder was not accounted for by cysteine, cystine, thiourea, thiocyanate, thiosulphate, urochrome, or melanin.

Estimations were made of the daily fluctuations in the quantity of thiouracil in the blood and urine of individuals maintained on standard dosages of the drug. Frequent small doses maintained a greater constancy in the blood and urine levels than did equivalent daily amounts administered less frequently. However, with all systems of dosages employed a distinct lability in the concentrations was observed.

The fetuses of rats given 0.1 per cent thiouracil in the drinking water during pregnancy were found to contain significant quantities of thiouracil. The small size of the fetuses as well as their goiters attested to the physiological activity of the drug *in utero*.

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The Control of Induced Estrogen Bleeding

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The use of male and female sex hormones in the treatment of the climacteric is well established, although it is recognized that each possesses a major disadvantage. For the estrogenic hormone this is abnormal uterine bleeding, while in the case of the androgenic hormone it is masculinization.

It is the purpose of this communication to report the combined use of the two hormones as a successful means of climacteric control, without the disadvantages of either abnormal bleeding or masculinization. A rationale is also considered for the occurrence of both phenomena. During the course of this study, covering a period of two years, Geist and Salmon (1) and Greenblatt (2) have reported the combined use of the two hormones in cases of induced estrogen bleeding.

PRELIMINARY STUDY

Although it is well recognized that the administration of estrogen causes bleeding in some instances, the importance of this fact is generally overlooked (3). As a review of the literature revealed little precise information as to the incidence of induced estrogen bleeding, the author made a preliminary study in an attempt to evaluate this quantity.

Abnormal bleeding was defined as bleeding in excess of, of greater duration than, or of greater frequency than, the usual menstrual flow of that patient. This definition includes many cases not usually so classified, where the excess or increased duration is only slightly greater than the usual menstrual flow. For example, if the usual menstrual flow is three to four days, and after estrogen administration it is five or more days, the administered estrogen is considered responsible. Or, if the duration is unchanged, but the amount of flow is noticeably increased, this also is defined as induced bleeding. This definition narrows the gap usually considered to exist between normal bleeding and menorrhagia.

This sharpening of the definition is, of course, important if the role of the various hormones in the production of uterine bleeding is to be understood. A determination of the true incidence of induced estrogen bleeding is not practical since the incidental occurrence of meno-metrorrhagia is difficult to evaluate. The present study gives not the true but the greatest possible incidence.

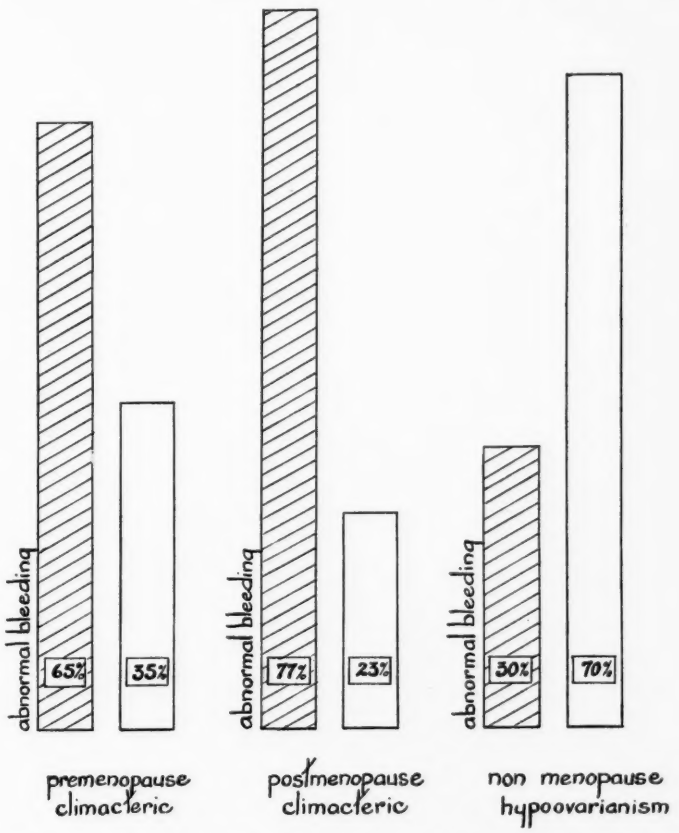
The preliminary study was made upon 45 cases. These were under observation for a period of at least one year and had sufficient estrogen administered to relieve symptoms and to produce a normal vaginal smear. These 45 cases consisted of 26 pre-menopausal climacteric, nine post-menopausal climacteric and 10 hypoövarian cases. The accompanying figure gives a graphical representation of the percentage of each group having abnormal bleeding.

COMBINED TREATMENT

Twenty of the cases presenting abnormal bleeding after estrogen administration were given combined treatment. For parenteral administration, where diethylstilbestrol could be tolerated, this was given with testosterone propionate. Where diethylstilbestrol was not tolerated, estradiol dipropionate was used with testosterone propionate. Either combination was mixed in a single syringe and given intramuscularly. For oral administration, diethylstilbestrol and methyl testosterone were prescribed as a single capsule. This later was changed to diethylstilbestrol, orally, and methyl

testosterone sub-lingually, when the latter became available. The results of both treatments are summarized in the table.

A specific ratio of androgen to estrogen, causing neither bleeding nor masculinization, was found to exist for each individual. In general the variation between different individuals was not marked; thus, specific ratios were (*27*) the ratio 0.1 mg. estradiol dipropionate to 25 mg. testosterone propionate was immediately followed by bleeding on several occasions. In this instance the administration of testosterone propionate alone not only inhibited bleeding, but did not cause the slightest degree of masculinization. Where estrogen caused slight degrees of abnormal bleeding, it was



The incidence of abnormal bleeding induced by the administration of sufficient estrogen to control symptoms and produce a normal vaginal smear.

available to satisfy a majority of cases. For those cases tolerating the synthetic hormone, the ratio of 10 mg. testosterone propionate to 5 mg. diethylstilbestrol did not induce bleeding in 95% of cases. In those not tolerating diethylstilbestrol, the ratio of 0.5 mg. estradiol dipropionate to 10 mg. testosterone propionate caused no bleeding in 88%. Masculinization never occurred in these instances. In one case found necessary only to add small amounts of testosterone propionate to the estrogenic hormone to control the bleeding.

Initially, the oral administration of androgen-estrogen combination was found to require relatively large amounts of methyl testosterone in order to prevent induced bleeding from small amounts of diethylstilbestrol. This was due to the greater oral effectiveness of diethyl-

TABLE SHOWING BLEEDING RESULTING FROM ESTROGEN ADMINISTRATION AND COMBINED ANDROGEN: ESTROGEN ADMINISTRATION
(With Control of Symptoms and the Production of a Normal Vaginal Smear)

	Estrogen Treatment		Combined Treatment			Results	
Case No.	Material, Amount and Mode of Administration	Duration of Administration	Material, Amount and Mode of Administration	Duration of Administration	Usual Menses Duration Amount	Abnormal Bleeding After Estrogen	Abnormal Bleeding After Combined Treatment
1	Diethylstilbestrol (P) 25 mg. Diethylstilbestrol (O) 0.25 mg. d.	17 days 6 weeks	Testosterone Propionate 10 mg. (480 mg.)[1] (P) + Estradiol dipropionate (0.5 mg. (24 mg.) (P)	6 months	7 days—xxx	7 weeks—xxxx Controlled with Testosterone Propionate (P) 220 mg.	0
7	Diethylstilbestrol (O) 1.0 mg. d Diethylstilbestrol (O) 1.5 mg. d	3 months 1 month	Diethylstilbestrol (O) 1 mg. d+ Methyl Testosterone (O) 20 mg. d. *or* Methyl Testosterone (sub-L) 5 mg. d.	8 months	4 days—x	9 days—xxx 28 days—xx 24 days—xx Controlled with Testosterone Propionate (P)	0
8	Diethylstilbestrol (O) 0.75 to 1.25 mg. d.	4 months	Diethylstilbestrol (O) 0.75 to 1.0 mg. d.+ Methyl Testosterone (O) 25 mg. *or* Methyl Testosterone (sub-L) 10 mg. d.	4 months	4 days—xx	7 days—xxxx Controlled with Testosterone Propionate (P) 100 mg.	0
10	Diethylstilbestrol (O) 0.5 to 2.0 mg. d.	3 months	Diethylsbilbestrol (O) 0.5 to 1 mg. d.+ Methyl Testosterone (O) 15–20 mg. *or* Methyl Testosterone (sub-L) 5 mg.	8 months	2 days—x	6 weeks—xx Controlled with Testosterone Propionate (P) 150 mg.	0
11	Diethylstilbestrol (O) 0.5 to 2.0 mg. d.	14 weeks	(1) Estradiol dipropionate (P) 0.2 mg.–0.5 mg. (19 mg.)+ Testosterone Propionate (P) 10 mg. (80 mg.) (2) Diethylstilbestrol (O) 0.5 mg. d.+ Methyl Testosterone (O) 30 mg. d. (3) Methyl Testosterone (O) 15 mg. d.	(1) 6 weeks (2) 6 weeks (3) 8 months	2 days—x–xxx	10 days—xxxx Controlled with Testosterone Propionate (P) 75 mg.	(1) 24 days—x, Controlled with Testosterone Propionate (P) 200 mg. (2) 13 days—xx, Controlled with Testosterone Propionate (P) 100 mg. (3) O
15	Diethylstilbestrol (P) 260 mg.	4 months	Diethylstilbestrol (P) 5 mg. (1115 mg.)+ Testosterone Propionate (P) 10 mg. (230 mg.)	4 months	Post-menopause	7 days—xx	0
17	Diethylstilbestrol (O) 1.0–4.0 mg. d.	4 months	(1) Diethylstilbestrol (O) 1.0 to 0.25 mg. d. +Methyl Testosterone (O) 20 to 30 mg. d. (2) Testosterone Propionate (P) 25 mg. (825 mg.)	(1) 3 months (2) 6 months	7 days—xxx	30 days—xxx	(1) 24 days—xx (2) 0
18	Theelin Aqueous (P) 14 mg.	30 days	(1) Theelin Aqueous (P) 2 mg. (46 mg.)+ Testosterone Propionate (P) 25 mg. (575 mg.) (2) Estradiol Dipropionate (P) 1.0–0.5 mg. (15 mg.)+Testosterone Propionate (P) 25 mg.–10 mg. (280 mg.)	(1) 6 months (2) 6 months	Post-menopause	20 days—x	(1) 0 (2) 0
19	Diethylstilbestrol (O) 0.5–1.5 mg. d.	2½ months	(1) Diethylstilbestrol (O) 1.0–0.5 mg. d.+ Testosterone Propionate (P) 25 mg. (675 mg.) (2) Testosterone Propionate (P) (100 mg.)	(1) 5½ months (2) 1 month	Post-menopause	2–7 days—x (4 times)	(1) 9 days—x (3 times) (2) 0
22	Estradiol Dipropionate (P) 28 mg.	5 months	Estradiol Dipropionate (P) 0.5–0.2 mg. (6.8 mg.) +Testosterone Propionate (P) 10 mg. (290 mg.)	10 months	2 days—x	6 days—xxx	0
26	Diethylstilbestrol (O) 1.0–5.0 mg. d.	6 months	Diethylstilbestrol (O) 0.25–1.0 mg.+ Methyl Testosterone (O) 20–30 mg. *or* Methyl Testosterone (Sub-L) 2.5–5 mg. d.	9 months	4 days—xx	14 days—xx 6 weeks—xxx Controlled with Testosterone Propionate (P) 180 mg.	0

TABLE SHOWING BLEEDING RESULTING FROM ESTROGEN ADMINISTRATION AND COMBINED ANDROGEN : ESTROGEN ADMINISTRATION—*continued*
(With Control of Symptoms and the Production of a Normal Vaginal Smear)

	Estrogen Treatment		Combined Treatment			Results	
Case No.	Material, Amount and Mode of Administration	Duration of Administration	Material, Amount and Mode of Administration	Duration of Administration	Usual Menses Duration Amount	Abnormal Bleeding After Estrogen	Abnormal Bleeding After Combined Treatment
27	Diethylstilbestrol (O) 1–3 mg. d.	4½ months	(1) Estradiol dipropionate (P) 0.5 mg. (0.5 mg.) +Testosterone Propionate (P) 10 mg. (10 mg.) (2) Estradiol dipropionate (P) 0.2 mg. (0.4 mg.) +Testosterone Propionate (P) 10 mg. (20 mg.) (3) Estradiol dipropionate (P) 0.2 mg. (0.2 mg.) +Testosterone Propionate (P) 25 mg. (25 mg.) (4) Testosterone Propionate (P) 25 mg. (850 mg.)	(1) 1 day (2) 5 days (3) 1 day (4) 7½ months	1 to 3 days x–xxx	9 days—x 6 weeks—xxx Controlled with Testosterone Propionate (P) 150 mg.	(1) 19 days—xxx Controlled by Test. Prop (P) 150 mg. (2) 8 days—xxx Controlled by Test. Prop (P) 50 mg. (3) 5 days—xx Controlled by Test—Prop (P) 25 mg. (4) 0 Normal menses occurred.
28	Diethylstilbestrol (O) 0.5–3 mg. d.	1 year	Diethylstilbestrol (O) 0.5 mg. d.+ Methyl Testosterone (O) 15 mg. d.	3 months	4 days—x	5 days—xx 6 days—xx 27 days—xxxx Controlled by Testosterone Propionate (P) 125 mg.	0
29	Diethylstilbestrol (P) 5 mg. (260 mg.)	4½ months	Diethylstilbestrol (P) 5 mg. (120 mg.)+ Testosterone Propionate (P) 10 mg. (240 mg.)	5½ months	2 days—x	10 days—xxx 7 days—xxx	0
30	None	—	Estradiol dipropionate 1.0 mg. (66 mg.)+ Testosterone Propionate (P) 25–10 mg. d. (1260 mg.)	9 months	5 days—xxx	—	0
32	Estradiol dipropionate (P) 2.5–1 mg. d. (53 mg.)	3½ months	Diethylstilbestrol (P) 5 mg. (120 mg.)+ Testosterone Propionate (P) 10 mg. (240 mg.)	5 months	Post-menopause	5 days—xx 10 days—xxx	0
33	None	—	Estradiol Dipropionate (P) 0.5 mg. (27.5 mg.) +Testosterone Propionate (P) 10 mg. (570 mg.)	10 months	4 days—x	—	0
34	Diethylstilbestrol (O) 0.25–0.75 mg. d.	2 months	Diethylstilbestrol (O) 1.0–2.0 mg. d.+ Methyl Testosterone (O) 20–30 mg. d. *or* Methyl Testosterone (sub-L) 5–10 mg. d.	9 months	Post-menopause	15 days—x	0
35	Estradiol dipropionate (P) 32 mg.	4 months	(1) Diethylstilbestrol (O) 1.0 mg. d.+ Testosterone Propionate (P) 25 mg. (625 mg.) (2) Diethylstilbestrol (P) 5 mg. (75 mg.)+ Testosterone Propionate 10 mg. (195 mg.) (3) Diethylstilbestrol (O) 0.75 mg. d.+ Methyl Testosterone (O) 20 mg. d.	(1) 5 months (2) 2 months (3) 6 months	5 days—xx	19 days—xxx Controlled with Testosterone Prop. (P) 225 mg.	(1) 0 (2) 0 (3) 0
36	Estradiol Dipropionate (P) (8.5 mg.)	6 weeks	Estradiol dipropionate (P) 0.5 mg. (32.5 mg.) +Testosterone propionate (P) 10 mg. (630 mg.)	9 months	Post-menopause	20 days—xxx Controlled with Testosterone Prop (P) 100 mg.	0
51	Estradiol dipropionate (P) 10 mg.	2 months	Estradiol dipropionate (P) 0.5 mg. (19 mg.) + Testosterone Propionate (P) 10 mg. (380 mg.)	6 months	4 days—xx	9 days—xxx	0

(P)—Parenteral
(O)—Oral
(Sub-L)—Sub-lingual.
d—daily

x—light bleeding (1–5 pads)
xx—moderate bleeding (6–12 pads)
xxx—heavy bleeding (12–20 pads)
xxxx—very heavy bleeding (20+pads)

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stilbestrol. On the average, it was necessary to use 25 mg. daily of methyl testosterone to 1 mg. diethylstilbestrol. It was found when the use of methyl testosterone was restricted because of cost, that bleeding was not controlled. During the course of the investigation, sublingual methyl testosterone became available, and was used in place of the oral tablet.[1] It was found to be much more satisfactory, 5 to 10 mg. daily sufficing for the administration of up to 1.5 mg. daily diethylstilbestrol, orally.

RESULTS

In every case it was found possible to determine a ratio of androgen: estrogen dosage which controlled both bleeding and masculinization. The 20 cases were all treated successfully.

Vaginal smears were made, routinely, on all patients. Those patients receiving estrogen alone would occasionally show a persistent estrogenic phase. Those receiving androgen alone required careful checking for signs of an atrophic phase. The 20 cases receiving the combined treatment never showed either of these characteristics.

The clinical results in climacteric control were more satisfactory than when either component was used alone in higher dosage. The majority of patients reported a greater feeling of well-being on combined treatment than when either hormone was administered alone. This may explain why some patients not obtaining relief from climacteric symptoms with estrogen administration may do so with androgen. The combined treatment eliminates the disadvantage of either hormone used singly.

THEORETICAL CONSIDERATIONS

Pathologic bleeding and menstruation are both due to fluctuations in the ovarian hormone level. It is believed that the endometrium is not a factor in uterine bleeding since such bleeding may occur from a progestational, hyperplastic, persistent estrogenic or atrophic endometrium (4). Further, a hyperplastic or persistent estrogenic endometrium may remain for many months after implantation of androgen pellets, although bleeding is controlled (5). It is considered that the myometrium and its vascular supply are responsible for uterine bleeding (6) and these are dependent upon endocrine control. The mechanism of the endocrine control is at present not understood.

As a rationale, it is here postulated that such endocrine control is exerted by means of a specific androgen: estrogen ratio in which androgen is the factor which constricts the myometrial portion of the spiral arterioles. With this hypothesis, a great variation in the incidence and amount of induced estrogen bleeding would be expected. Women with a high androgen level could receive estrogen overdosage without bleeding, but would respond readily to androgen with masculinization. Those with a low androgen level would bleed with inadequate estrogen dosage, but would become masculinized with difficulty. Of course there may be other factors. Normal liver function and the presence of an adequate Vitamin-B level are necessary for the inactivation of estrogen (7). Thus, B-avitaminosis may lead to the accumulation of estrogen so that a higher blood level results than would be expected from the amount of estrogen administered. This may be an indirect factor in disturbing the androgen: estrogen balance and allowing abnormal bleeding to occur.

DISCUSSION

If the preceding rationale is correct, the aim in treatment of the climacteric should be to maintain a normal androgen: estrogen ratio. In any event, the present report indicates that as an empirical measure this procedure has been successful in preventing two of the commonest complications of climacteric treatment. Extremes of imbalance occasionally occur and examples have been encountered in the series studied. One patient received an average of 5 mg. diethylstilbestrol, orally, per day for six months without abnormal bleeding. This represents a high androgen, low estrogen level. A low androgen, high estrogen ratio is exemplified by the patient who responded on several occasions to 0.1 mg. estradiol dipropionate plus 25 mg. testosterone propionate with flood-

[1] Thanks are due to Mr. Angehrn of the Ciba Company, Canada, for supplying the sub-lingual methyl testosterone (Metandren) used in this study.

ing, but who was not masculinized on testosterone alone. In these cases the administration of the single hormone is the means of maintaining a normal androgen: estrogen ratio. The proper ratio can readily be determined by the addition of just sufficient androgen to the administered estrogen to prevent bleeding. Such ratios will never cause masculinization. It is recognized that, in part, the control of bleeding and masculinization is due to the fact that, together, each hormone is used in lesser dosage than when employed singly.

It may be noted in the accompanying figure that the incidence of abnormal bleeding is much less in the hypoövarian group although treated with similar amounts of estrogen. This suggests that the menopausal state includes a variation in androgen level as well as in estrogen decline, while the hypoövarian state represents, primarily, a decreased estrogen level.

SUMMARY

Estrogen and androgen used in the treatment of the climacteric have the respective disadvantages of abnormal uterine bleeding and masculinization. The present study reports the combined use of the two hormones as successful treatment without either disadvantage.

Various ratios of androgen to estrogen existed for individuals but for a majority of cases were as follows: for parenteral administration, 10 mg. testosterone propionate to 0.5 mg. estradiol dipropionate or 5 mg. diethylstilbestrol, where this could be tolerated; for oral administration, 5 mg. daily methyl testosterone, sub-lingually, to 1.0 mg. daily diethylstilbestrol. In a minority of cases these ratios were altered in either direction and in either case might become zero.

A rationale for the combined treatment is suggested in which androgen is the factor which controls the extent of uterine bleeding by constricting the myometrial portion of the spiral arterioles and estrogen is the factor which limits the masculinizing influence of androgen.

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Observations on the Experimental Use of Gonadotropic Extracts in the Human Female[1,2]

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Recent reviews have focused attention on the fact that the available gonadotropic preparations have provided little therapeutic aid. Chorionic gonadotropins are of questionable value in the human female. Although equine gonadotropin resembles the anterior-pituitary lobe in its action, its role in man is still debatable. Chorionic gonadotropins can produce follicle growth and ovulation in laboratory animals but the human being is not responsive to its action. Extracts of the anterior-pituitary lobe provide the greatest possibility for the future.

Pituitary gonadotropins have been used in the laboratory for many years. Implants and crude extracts of the anterior lobe have replaced the action of the pituitary in hypophysectomized animals. These impure preparations could not be used in the human. It is only recently that methods of purification have progressed to the point where pituitary extracts could be administered to women without serious hazards.

Casida and his co-workers have studied the action of pituitary gonadotropins on calves, cows, sheep and other animals since 1934. Their studies embrace the entire reproductive cycle. It has been possible to produce follicle growth, maturation, ovulation and corpus luteum formation. Super-ovulation occurred in many of the animals. Ova discharged from follicles were normal in that they could be fertilized and implanted within the uterus. Although it was possible to produce multiple implantations, most of the embryos died after varying periods of development. It was possible to produce seven implantations in the cow and the embryos developed normally for a period of time. Nevertheless, it was impossible to increase the normal number of young by artificial ovulations produced by gonadotropins. Casida and his associates assumed that this was due to the inability of the uterus to accommodate itself to more than the normal number of young. Uterine environment rather than abnormal ova resulting from gonadotropic stimulation was blamed for the abnormally high death rate of early embryos.

Extracts. The pituitary gonadotropins used in this study were provided by R. K. Meyer and W. H. McShan of the University of Wisconsin. They were prepared according to methods described in their several publications. Briefly, the extracts were prepared from acetone desiccated sheep pituitary powder. The follicle-stimulating extracts were prepared from aqueous extracts by digestion with trypsin. The trypsin is removed from the digested extracts by heat treatment and further purification is effected by dialysis. Luteinizing, lactogenic and thyrotropic activities are destroyed. Meyer and McShan report the following changes in their trpytic digestion

[1] Read before the Twenty-Seventh Annual Meeting of the Association for the Study of Internal Secretions, Chicago, Illinois, June 12, 1944.

[2] This work has been done under a grant from the Douglas Smith Foundation for Medical Research of The University of Chicago.

method described in 1940; they were instituted to remove local irritating factors so that the material could be used in the human. These changes in the method can be summarized briefly.

The pH 4 soluble digest was dialyzed against distilled water, centrifuged and the active material precipitated with ethyl alcohol. The hormone was extracted from this precipitate with distilled water and again precipitated with acetone. The active principle was collected and dissolved in a solution containing 0.7 per cent sodium chloride and 0.02 M phosphate of pH 7.4. The solutions of the hormone that were used in this study contained 4 gram-equivalent of acetone-desiccated sheep pituitary powder per cubic centimeter.

The fractionated (FSH) extracts were assayed on 21-day-old rats, using 0.5 and 1.0 gram-equivalent dosages. The ovarian weights can be seen in table 1. The ovaries of the test animals contained only follicles. The unfractionated extracts were prepared from sheep pituitary powder by the elimination of inert material from the aqueous extract. The gonadotropin is obtained free of lactogenic hormone. When assayed on rats in the same way as were the fractionated (FSH) extracts, they produced follicle growth, luteinization and corpora lutea. Table 2 presents the assays of the unfractionated preparations used in this study.

The dosages have been expressed in gram equivalents. This refers to the number of grams of the original acetone-desiccated powder which had been extracted and were represented in the dosage.

The two types of extracts were used in this study. The unfractionated material contains

TABLE 1. ASSAY OF FRACTIONATED (FSH) PREPARATIONS USED SINCE SEPTEMBER 1943
(Number of rats used in each assay is given in parentheses)

No.	Av. Weight of Ovaries Obtained on Assay of FSH using Doses of:		Qualitative Response
	0.5 gm. equiv.	1 gm. equiv.	
FSH234	38 (6)	90 (6)	All follicles
FSH235	50 (6)	94 (3)	All follicles
FSH236	68 (3)	90 (3)	All follicles
FSH320	42 (3)	87 (3)	All follicles
FSH321	35 (3)	104 (3)	All follicles
FSH322	46 (6)	—	All follicles

The ovaries of the untreated control rat average 12 mg.

both follicle-stimulating and follicle-luteinizing activity. The fractioned extracts are predominantly follicle-stimulating. Some of the earlier preparations produced reactions when administered subcutaneously or intramuscularly. These consisted of redness, some local swelling and tenderness at the site of injection. Later preparations were well tolerated by the patients. Unfractionated extracts were administered intravenously very guardedly. A mild rise in temperature occurred in several

TABLE 2. ASSAY OF UNFRACTIONATED (F) PREPARATIONS USED SINCE SEPTEMBER 1943
(Number of rats used in each assay is given in parentheses)

No.	Dose	Av. Weight of Ovaries	Qualitative Response
	mg. equiv.		
F122C	100	82 (6)	Corpora
F122G	100	64 (6)	Corpora
F122F[1]	500	107 (3)	Corpora

[1] Weak preparation.

instances. A few women complained of transitory faintness. No untoward complications developed. All of the patients were carefuly skin-tested for protein sensitization by the intradermal administration of minute amounts of the extracts. Although the preparations were put through a Seitz filter their sterility was assured by the addition of a small amount of merthiolate.

Nature of Study. No methods comparable to the usual animal studies for evaluating the several extracts are available in the human. Clinical observations can be made in women with normal and abnormal ovarian activity before and after the use of these extracts. Their effects on the normal cycle as well as their ability to correct abnormalities could interpret their efficacy. However, pituitary failure is difficult to ascertain and the proper selection of cases impossible. The second method is the study of gross and microscopic ovarian changes. The gonadotropins available for clinical use at the present time produce no consistent changes in the ovaries. The ability of gonadotropic extracts to produce follicle growth, ovulation and corpus luteum formation are accurate indices of their activity. However the patient's own intact pituitary must be considered in the interpretation of any findings. The state of the ovaries prior to the use of an extract is difficult or impossible to determine.

The primary objective of this study was to determine the effect of pituitary gonadotropic

extracts on the ovaries of selected patients. The first group were recently delivered mothers in whom it was necessary to ligate the Fallopian tubes to prevent future conception. These patients were injected once or twice a day for 10 or 12 days following delivery. The ovaries were inspected at laparotomy and one of them removed for study. No complications followed selected primarily because patients were available in whom it would be possible to inspect the ovaries and remove one for gross and histologic examination. Furthermore, previous studies had revealed the typical changes in the ovaries at this time, thereby providing a group of controls for this study. Little follicle activity occurs in the human ovary during pregnancy

TABLE 3. WOMEN INJECTED INTRAMUSCULARLY WITH PITUITARY GONADOTROPINS

Unit No.	Age	Days P.P.	Extract Type and Number	Tot. Gm. Equiv.	No. of Inj.	Days before Operation	Size of Ovary cm.	Diam. of Av. Fol. mm.	Remarks
P.P. 297214	31	2	UNF F122	20.5	11	9	3.5×2	6	Grossly typical of postpartum ovary. Atretic follicles lined by 3–4 layers granulosa cells.
185516	22	3	UNF F122	24	20	12	5×3	10	Ovary almost completely replaced by large follicles containing a gelatinous substance which coagulates. Walls consist of 6–7 layers of active granulosa cells.
303696	26	1	UNF F121A	27	18	12	8×5	10–12	Whole ovary converted into follicle cysts. Large cyst lined by 5–6 layers of granulosa cells
214894	27	1	UNF F121A	25	16	10	3.5×2	5	Follicles lined by 4–5 layers of granulosa cells.
305388	29	5	UNF F122A	45	19	13	3.5×4	5	Follicles of various sizes, most of them atretic. Few small follicles show active growth.
53321	31	1	UNF F122B FSH 234	105 24	15 3	13	5×4.2	12-14	Large follicles. Some atretic; others show growth of granulosa cells.

these experimental procedures. The second group of patients were pregnant but the therapeutic terminations of these pregnancies were necessitated by the presence of serious accidental diseases. These women were injected with gonadotropins and the pregnancy terminated at the desired interval following the administration of the extracts. The ovaries were inspected and one was removed for histologic study. The third group of women entered the hospital with gynecologic complications necessitating laparotomy. They provided an opportunity to inspect and remove an ovary for study at the proper interval following the administration of the extracts. The uterus was likewise available for study in many of these cases.

Gonadotropins During the Immediate Postpartum Period. The postpartum period was and the early puerperium. Follicles develop to a moderate degree but these undergo slow retrogression, the ovum disappearing and the follicle becoming atretic. None of the follicles reach a sufficient degree of development necessary for ovulation. With the exception of the corpus luteum the ovaries do not increase in size nor in weight but become somewhat flattened and elongated. The cross section of an ovary removed 24 hours to two weeks after delivery reveals numerous small, atretic follicles in the cortex arranged rather symmetrically beneath the superficial surface of the ovary. These atretic follicles vary in size but the larger ones are quite uniform.

Six women received varying amounts of unfractionated extracts in divided doses (table 3). The initial injections were started in the immediate postpartum period. The patients were

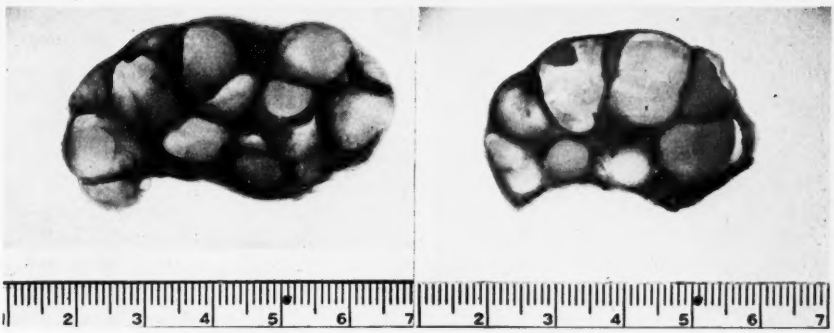

FIG. 1. (Left) Unit No. 303696. Translucent appearance of a cross section of the ovary removed 12 days postpartum following the injection of a total of 27 grams equivalent of unfractionated extract intramuscularly. (Scale in centimeters.)
FIG. 2. (Right) Unit No. 185516. Translucent appearance of a cross section of the ovary removed 12 days postpartum following the injection of a total of 24 grams of unfractionated extract intramuscularly.

operated upon 9 to 13 days after the first injection. The size of the ovaries varied in a great measure with the total amount of the extract administered, although in patient number 303696, who received a total of 27 grams equivalent, the ovaries were huge, weighing 15 times as much as the normal ovary. The follicles varied greatly in size. Many appeared to be atretic but others had 6 or 8 layers of normal active granulosa cells. They contained a clear fluid which jelled when the ovary was placed in a fixing solution. No new corpora lutea were evident in any of these ovaries.

Casida and his co-workers found that they obtained the best results when animals were treated with fractionated extracts subcutane-

TABLE 4. WOMEN TREATED BY FRACTIONATED EXTRACTS (F.S.H.) FOLLOWED BY UNFRACTIONATED EXTRACTS (L.H.) DURING THE POSTPARTUM PERIOD

Unit number	Intramuscular Injection					Intravenous Injection							Remarks
	Age	P.P. Day	Extract Type and Number	Total Gm. Equiv.	No. of Injections	Extract Type and Number	Total Gm. Equiv.	Days after Intramuscular	No. of Injections	Days before Operation	Size of Ovary Cm.	Diameter of Av. Follicle Mm.	
259064	27	1	FSH 529	32.5	17	UNF 121A	1.5	1	1	14			Few small follicles. Little change in ovaries.
249163	23	5	FSH 529	46	19	UNF 121A	3	1	1	14			Most follicles are atretic. Occasional one shows active granulosa cell layer.
244409	34	3	FSH 235	87.5	16	UNF[1] F122C	15		4	12	4.3×2.4	12–14	Large follicle cysts lined with 6–8 layers small granulosa cells.
131700	26	4	FSH 231B	109.6	14	UNF[1] F122C	15		6	14	3×4	8–10	Large follicles lined by about 6 layers of granulosa cells. One of smaller follicles shows a normal ovum.
226991	28	2	FSH 235	105	17	UNF[1] 122A	10		3	12	3.5×2		Most follicles lined with 5–6 layers of active granulosa cells.
185553	40	3	FSH[1] 231& 529	126.7	18	UNF[1] F122B	15		2	14	2.5×2		Almost no change in ovary.

[1] Intramuscular injection.

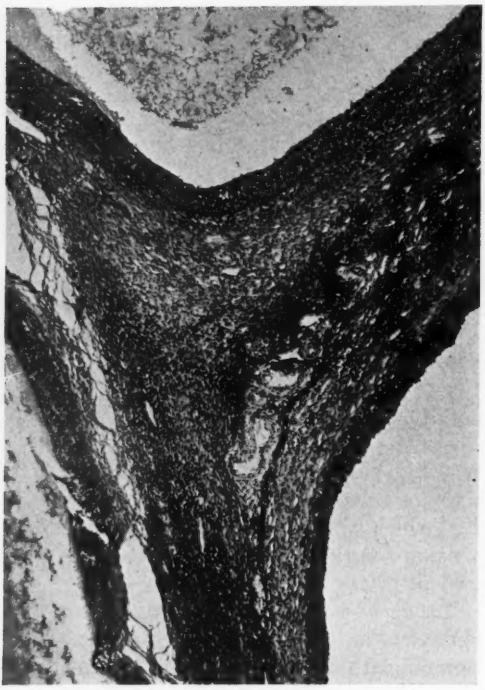

FIG. 3. Unit No. 53321. The histologic appearance of portions of the walls of several follicles in an ovary removed on the 13th postpartum day following intramuscular injections of an unfractionated extract. (80×)

ously followed by the intravenous administration of an unfractionated extract. In our early work we were reluctant to administer these gonadotropins intravenously. Table 4 presents a summary of data on four patients to whom the unfractionated extract was administered intramuscularly and two to whom it was administered intravenously. Follicles were stimulated to grow and many large follicles were present. However, ovulation was not produced in any of these patients.

It is possible that the postpartum period in women presents a poor time for gonadotropin evaluation for the ovaries may be refractory to normal stimulation. However, it has been possible to produce follicle growth and the numerous follicles of large size and their normalcy would indicate that the puerperal ovary can be stimulated. The most likely explanation for the failure to produce ovulation and corpora is to be found in the dosage of the extracts and in the timing of their administration.

Gonadotropins in Gynecologic Patients. Table 5 summarizes the results obtained in three normal young women. The injections began early in the cycle and they were operated upon 14 days later. In two of the patients huge ovaries were produced and moderate sized ovaries in the third woman. The follicles varied from 10 to 20 mm. in diameter and were lined by 8 to 12 layers of normal granulosa cells. Most of the large follicles seemed normal except for their size. No recently ruptured follicles or corpora lutea could be demonstrated.

Data on three patients who received fractionated extracts intramuscularly followed by the intravenous administration of an unfractionated extract are given in table 6. The ovaries contained many maturing follicles but the response was not as great as in the women who received unfractionated extracts only. It is possible that a sufficient amount of unfractionated material was not administered, for, at the suggestion of Casida, a total of 15 grams equivalent was given in four divided doses. This amount is sufficient to produce ovulation in the cow but it may not represent a sufficient stimulus in the human.

Gonadotropins During Pregnancy. Eight women received unfractionated gonadotropins at varying periods during gestation (table 7). The amounts varied from 17 to 97.5 grams

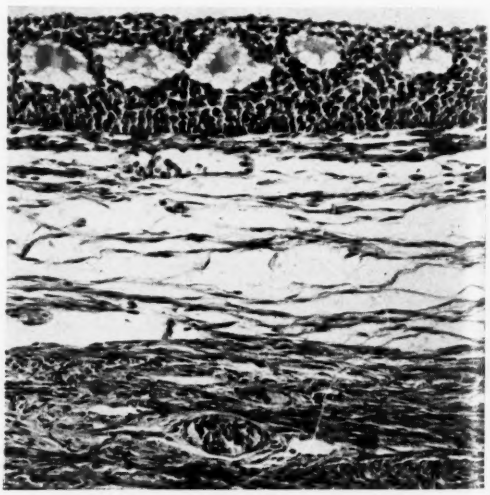

FIG. 4. Unit No. 185516. The histologic appearance of the granulosa cells lining the walls of most of the large follicles in the ovary illustrated in Fig. 2. (225×)

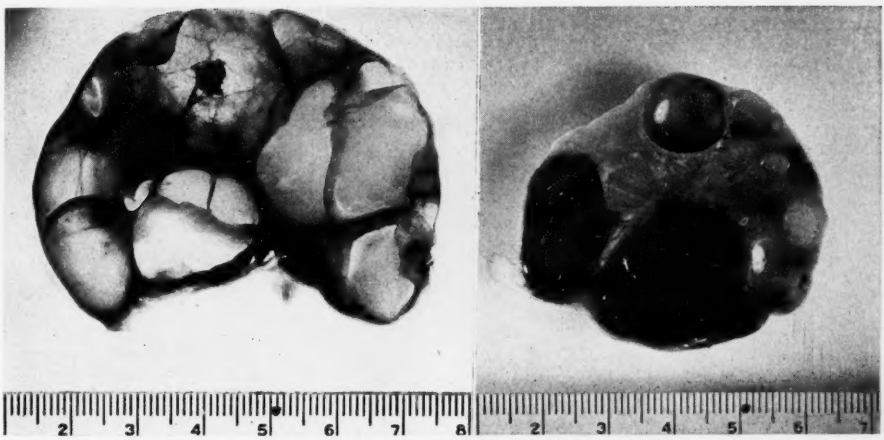

FIG. 5. (Left) Unit No. 305662. Translucent appearance of a cross section of the ovary removed from a normal, 28-year-old woman. Injections of unfractionated gonadotropins intramuscularly began on the 9th day of the cycle and the ovary was removed on the 23rd day.

FIG. 11. (Right) Unit No. 49963. A gross cross-section of an ovary removed from a woman, 16 weeks pregnant, who was injected with 97.5 grams equivalent of an unfractionated extract intravenously. Several large, discrete hemorrhagic follicles are visible.

equivalent in divided doses. Pregnancy was not interfered with in any of these patients. The ovaries increased in size greatly; the gross response equalled or exceeded that obtained in the postpartum group of women. Many follicles reached diameters of 12 to 15 mm. and their thin walls were easily bruised. Marked vascularity was present superficially about the follicles as well as within the ovary. In two patients hemorrhagic follicles were present. The hemorrhage was confined to the cavity of discrete follicles and did not involve contiguous stroma. Histologically, all degrees of follicle development were apparent. The large follicles were lined by 8 to 12 layers of normal granulosa cells. The ovum was visible in many instances. In the last three patients listed in table 7 the removed ovary contained a collapsed follicle which appeared to have ruptured recently. The walls were lined by branching columns of granulosa cells. The cavities contained a small amount of blood.

Three pregnant patients received fractionated extracts intramuscularly followed by an unfractionated extract intravenously. The gross reaction was not as great as in the patients who received unfractionated extracts only. There was considerable follicle stimula-

TABLE 5. WOMEN INJECTED INTRAMUSCULARLY WITH PITUITARY GONADOTROPINS

Unit No.	Age	Day of Cycle	Extract Type and Number	Tot. Gm. Equiv.	No. of Inj.	Days before Operation	Size of Ovary cm.	Diam. of Av. Fol. mm.	Remarks
GYN. 300124	19	6	UNF F79	39.5	21	14	6×8	12–15	Most of large follicles lined by 8–10 layers of granulosa cells. Considerable hemorrhage in stroma.
305662	28	9	UNF F122A	28	20	14	6×5	20–30	Ovary converted completely into large cysts lined by several layers of cells.
194221	36	10	UNF F122A	78	11	14	2×3	12	Numerous follicles lined by normal granulosa cells.

TABLE 6. WOMEN TREATED BY INTRAMUSCULAR FOLLOWED BY INTRAVENOUS INJECTIONS OF PITUITARY GONADOTROPINS

	Intramuscular Injections					Intravenous Injection							
Unit number	Age	Day of Cycle	Extract Type and Number	Total Gm. Equiv.	No. of Injections	Extract Type and Number	Total Gm. Equiv.	Days after Intramuscular	No. of Injections	Days before Operation	Size of Ovary Cm.	Diameter of Av. Follicle Mm.	Remarks
GNY.													
179292	27	23	FSH 320	15	4	UNF F122C	10	1	1	7	5×4.5		Several new follicles.
167057	27	22	FSH 320	15	4	UNF F122C	3	2	1	9	4×3	3.5	Many normal follicles throughout ovary. Normal corpora lutea present.
326079	28	19	FSH 322	15	4	UNF F122F	5	2	2	9	5×5		
PRE.		*Wks. Gest.*											
259064	27	40	FSH 529	32.5	17	UNF F121A	1.5	1	1	14	6×1.8		Few small follicles. Little change in ovaries.
249163	23	40	FSH 529	46	19	UNF F121A	3	1	1	14	4×3		Most follicles are atretic. Occasional one shows an active granulosa cell layer.
253795	23	18	FSH 320	80	20	UNF F122A	6	1	2	13	4.5×3	5–7	Some follicles appear normal with a cumulus and ovum. Others are atretic.

TABLE 7. WOMEN INJECTED INTRAMUSCULARLY WITH PITUITARY GONADOTROPINS DURING PREGNANCY

Unit No.	Age	Wks. Gest.	Extract Type and Number	Tot. Gm. Equiv.	No. of Inj.	Days before Operation	Size of Ovary cm.	Diam. of Av. Fol. mm.	Remarks
261775	43	17	UNF F76	17	11	9	3×2.7	3	Normal corpus luteum. Most follicles atretic. Several appear stimulated.
216532	30	40	UNF F75	7.5	8	6		3	Number of large follicles. Others appear normal.
154187	31	38	UNF F79	21	14	9			Ovaries examined in situ. No marked stimulation.
200062	27	40	UNF F122	24	20	13	3.5×1.7	6	Most follicles are lined by 5–6 layers granulosa cells. Marked theca cell proliferation.
306977	27	8	UNF F121A	33	20	13	9×5	15–20	Many large follicles. Normal cystic corpus luteum.
241982	44	16	UNF F121A	34	18	14	3×1.7	12	Normal active corpus luteum. Recently ruptured follicle, quite hemorrhagic. Marked luteinization of granulosa cells.
49963	20	16	UNF F122A	97.5	20	13	6.5×5	10–15	Large hemorrhagic follicles. Granulosa appears normal. Recently ruptured follicle?
210929	36	12	UNF F122A	97.5	20	13	6×5	10–15	Numerous hemorrhagic follicles. One recently ruptured.

tion but not to the degree seen in the ovaries of women who were treated with unfractionated extracts only.

DISCUSSION

The human ovary can be stimulated by gonadoptropic extracts in non-pregnant women during their active reproductive years, as well as during pregnancy and in the immediate postpartum period. Follicles grow at an astonishingly rapid rate and rapidly pass from physiologic to pathologic states. Our present criteria do not make it possible to differentiate a normal from an abnormal follicle in the human but it is hardly conceivable that the huge follicles present in some of the ovaries were normal. Histologic appearances did not estab-

FIG. 6. Unit No. 241982. A low-power, histologic section through a portion of an ovary removed from a patient, 16 weeks pregnant, who was injected with 34 grams equivalent of an unfractionated extract. A recently ruptured follicle can be seen adjacent to the corpus luteum of pregnancy. (10×)

FIG. 7. Unit No. 241982. A high-power, histologic section of the wall of the large follicle illustrated in Fig. 6, showing the granulosa cells. (140×)

FIG. 8. Unit No. 210929. A low-power section through a portion of an ovary removed from a patient, 12 weeks pregnant, who was injected intramuscularly with 97.5 grams equivalent of an unfractionated extract. Several large follicles are visible, one of which has apparently ruptured. (8×)

lish always the normalcy of many of the follicles. The rapidity of ovarian enlargement and coincidental follicle growth is a striking observation.

The unfractionated extracts are more potent than the fractionated extracts. Casida and his co-workers concluded that normal ovarian changes could be induced most easily in animals by the development of follicles by the subcutaneous administration of fractionated extracts and their rupture and corpus luteum conversion accomplished by the intravenous use of unfractionated extracts which contain LH. In a human, this procedure was not successful. This may be the result of an inadequate dosage or because the unfractionated extracts administered intravenously were more purified and contained less luteinizer than that used by Casida.

The most encouraging results were obtained during pregnancy. In three of the eleven patients follicles appear to have ovulated just prior to laparotomy. It is possible that the hormones of pregnancy may have enhanced the gonadotropins injected in these patients. In all of this work one must not lose sight of the fact that an intact pituitary gland is present and may influence any changes induced by injected gonadotropins.

FIG. 9. Unit No. 20062. A low-power section through a portion of an ovary removed from a patient at the end of pregnancy, who was injected intramuscularly with 24 grams equivalent of an unfractionated extract. Many normal developing follicles are visible throughout the ovary. (6×)

FIG. 10. Unit No. 249163. A low-power section of an ovary removed from a woman at the end of pregnancy who was injected with 46 grams equivalent of fractionated extract followed by 3 grams equivalent of an unfractionated extract intravenously. (9×)

CONCLUSIONS

It has been possible to produce ovarian stimulation in women by fractionated and unfractionated extracts of sheep pituitary. The ovaries of the adult woman in the reproductive period, during pregnancy, and in the early puerperium are susceptible to gross gonadotropic stimulation. Although it was possible to produce follicle growth of all degrees and possibly follicle rupture in several instances, no corpora lutea were produced. Now that potent pituitary gonadotropins are available for experimental use in the human, further observations may result in our ability to produce readily artificial follicle growth, ovulation

and corpus luteum formation. This will increase our knowledge of the physiology of reproduction in the human as well as to aid in therapy of pathologic conditions of the reproductive period.

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EDITORIAL

RAPID TESTS FOR PREGNANCY

TESTS TO facilitate and expedite the diagnosis of pregnancy are legion and date back to the days of the Pharoahs. Egyptian priests, suspecting that urine from pregnant women had growth-stimulating properties, poured such samples onto certain seedlings and observed the relative rapidity of sprouting. It was not until 1928, however, that the first accurate laboratory test for pregnancy was devised. Aschheim and Zondek demonstrated the gonad-stimulating substances in the urine of pregnancy and an epoch in endocrinology was begun.

The need for simplification of the A.Z. test soon was felt. Many procedures, ranging from biological to chemical have been suggested. Of these, many endured but a brief span; others enjoyed some degree of popularity; few have withstood rigid clinical trial or the test of time. In 1931 the modification of the A.Z. test offered by Friedman and Lapham proved an important contribution. The original procedure was simplified and the employment of rabbits instead of immature mice permitted its general adoption for routine laboratory use. Furthermore, the time element was reduced from 96 to 48 hours without sacrificing accuracy.

The desire and need for reducing the time element in tests utilizing the common laboratory animals became a compelling force with many research workers. In 1940 Kelso (1), used the immature female rat as the test animal. The hyperemic effect on the ovary of anterior-pituitary-like substances in the urine of pregnancy was noted when the animal was sacrificed in 24 hours. Frank and Berman (2), in 1941, used the same "end-point" as Kelso and shortened his procedure to 8–24 hours. In 1942, Salmon *et al.*, (3) found that the "end-point" occurred more rapidly than was anticipated by Kelso or Frank and Berman and showed that the test could be read in 6 hours. In fact, Salmon *et al.*, (4) observed that the "end-point" was evident in 2 hours. When the test animals were killed in less than 6 hours the accuracy of the readings diminished in direct ratio to the reduction of the time element. Kelso, Frank and Salmon, all utilized the same "end-point" (hyperemia of the ovaries and ovarian capsule), the same method of injection (subcutaneous), and the same test-animal within a narrow range of age and weight. Recently Kline (5a) confirmed the reliability of the 6-hour rat test and, among other suggestions, advocated further limitation of weight range to 47–55 gm. and age range of 22–26 days. The validity of the two-hour rat test as performed by Salmon has been questioned by Farris (5b). In 1943, Kupperman *et al.* (6), by modifying the method of injection from subcutaneous to intraperitoneal, found that the "end-point" was apparent in 2 hours with maintenance of accuracy. Furthermore, by this modification, the weight and age range of the test animals could be greatly increased. The immature female rat, 21–55 days of age or 30–100 gm. in weight, proved satisfactory for the test (7). The intraperitoneal injection of urine into immature rats first was employed by Kelly in 1933. The "end-point" in this pregnancy test, however, was patency of the vagina (8).

In recent years, Weisman and Coates (9) have brought to the forefront in this country the spectacular frog test of Hogben. This test first was used extensively by Shapiro and Zwarenstein in South Africa (10). As the African clawed toad (Xenopus laevis) becomes more readily available, the test may enjoy a great degree of popularity, as much for its glamor as for its accuracy. The advantages of simplicity in the test on the Xenopus and the fact that the frogs may be reused indefinitely are offset by the necessity of concentrating the urine with acetone and adjusting the PH. Furthermore, though the extrusion of eggs, indicating a positive test, usually occurs within 6 to 8 hours after injection, 18 hours must elapse before the test may be declared negative. Recent work indicates that bright illumination of the test animals leads to "false positives." It is only fair to say that the test is not without many encumbrances. Other tests utilizing frogs and fishes, such as the female-bitterling test of Fleischman and Kann, the male-bitterling test of Tozawa, and the frog-melanophore test of Konsulov, have proved non-specific.

Several skin tests have been proposed for the diagnosis of pregnancy. These are based on the assumption that an allergic wheal is produced at the site of the injection when some particular substance that is common to pregnancy is introduced intradermally into the non-pregnant woman. The Gruskin test utilizes placental ex-

tract; that of Pollatschek and Porges employs chorionic gonadotropin; and the Falls-Freda (11) reaction uses colostrum. The last-mentioned test seemed most promising but the results have not been confirmed by independent investigators.

Chemical procedures have been proposed to circumvent the inconveniences of test animals. The Visscher-Bowman test, the histidine test of Kapellar-Adler, and the colorimetric test of Schmulowitz and Wylie depend on the color reaction produced by the addition of certain chemicals to the urine of pregnancy. None has proved accurate—particularly in the early months of gestation. More recently, Gutterman (12) made use of the observation by Venning and Browne (13) that pregnandiol is excreted in increasing amounts with the progress of pregnancy. A simple chemical procedure was devised by modifying Astwood and Jones' method for extraction of pregnandiol from urine and Talbot's colorimetric determination of the substance. The qualitative color-reaction so obtained is easy to interpret and Gutterman's results compared favorably with those of the Friedman test. The procedure is said to have the following advantages: (1) rapidity (3 hours); (2) does not involve the use of animals; (3) becomes positive earlier than the Friedman test; (4) in conditions other than pregnancy, in which the prolan excretion is raised sufficiently to give a positive Friedman reaction, the pregnandiol test is negative. An opportunity, as yet, has not been afforded to confirm the color test's value. Suffice it to say, it rests on a sound biochemical basis. The procedure is not simple and is rather laborious, consuming 3 hours of constant effort.

The general trend in tests for pregnancy has been to search for simplicity, specificity and speed. When urgency is not an important factor the Friedman test will continue to be used. When an immediate diagnosis is required—as in suspected ectopic pregnancy—minutes and hours, not days, are available for confirmatory evidence, rapid pregnancy tests hold a place of immeasurable importance. To this end it is well that a laboratory group develop proficiency in at least one of the following: the 2-hour rat test of Kupperman, the 6-hour rat test of Salmon, the 8- to 24-hour rat test of Frank or the frog test of Hogben. Perhaps the pregnandiol test of Gutterman will prove a worthy complement to one of the aforementioned rapid biologic tests to affirm or deny a diagnosis of pregnancy.

R.B.G.

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LETTER

THE AFRICAN FROG TEST FOR PREGNANCY

To the Editor

Our attention has been drawn to the increasing publicity given in the lay as well as the scientific press in the United States to the use of the South African frog (*Xenopus laevis*) as a test for pregnancy.

In all these recent communications there has been no adequate reference to our original report published in October, 1933 in the *Proceedings of the Royal Society of South Africa*.

In the interests of accuracy we feel it necessary to point out that this test is not being described for the first time, as it is already nearly 11 years old.

Because we feel sure that our American colleagues who are using the frog test have no desire to create confusion about the priority and authorship of this test, we wish to draw attention to the correct citation of the original reference.

The interested reader will find subsequent references in: *Nature*, 133: 762, 1934, as well as *South African M. J.*, 9: 202, 1935, i.e. some 3 years before Elkan first used the test in England and about 7 or 8 years before Weisman *et al.* first used it in America.

The most recent review of the test, summarizing 10 years' experience in this field, will be found in *Clinical Proceedings*, 3: 186, 1944.

H. A. Shapiro
H. Zwarenstein

University of Cape Town Medical School
Mowbray, Cape Town, South Africa
August 17, 1944

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: D. A. McGINTY. *Collaborators:* ISRAEL BRAM, RUCKER CLEVELAND, JOHN W. EVERETT, ANNE FORBES, ROBERT B. GREENBLATT, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, THOMAS H. MCGAVACK, A. E. MEYER, DORIS PHELPS, J. P. PRATT, E. C. REIFENSTEIN, JR., PATRICIA H. SMITH, RUTH ST. JOHN, CHARLES W. TURNER, AND HAROLD WOOSTER.

GONADS

STRAUSS, H. A., L. FISHER AND B. B. RUBENSTEIN

The effect of medical diathermy on the menstrual cycle of the monkey (Macacus rhesus). *Am. J. Obst. and Gynec.* 46: 861. 1943.

Four adult female macaque monkeys were subjected to daily examinations with regard to the menstrual cycle and ovulation. Vaginal smears and the rectal palpation technic of Hartman were used. Medical diathermy consisted of the conventional long-wave diathermy, using an intravaginal electrode. It was given at 30 milliamperes for 10 minutes each day, from the cessation of the menstrual flow and for 12 consecutive days, omitting Sundays. Two animals were treated at one time, the others serving as controls. There was a definite and consistent shortening of the menstrual cycles, increase in the duration of flow, and increase in number of ovulations. Similar clinical studies seem warranted.—*E.C.H.*

TALISMAN, M. R.

A clinical and laboratory study of the synthetic estrogenic substance, octofollin. *Am. J. Obst. and Gynec.* 46: 534. 1943.

Octofollin, a new synthetic, non-hormonal estrogen, was given to 21 patients who had had bilateral oöphorectomy. The dosage varied from five to thirty milligrams daily and treatment was started on the third or fourth post-operative day and continued for a varying length of time. Complete blood studies, urinalyses, blood pressure determinations, icterus indices were done before and during therapy. No evidence of any toxic changes was found. Intramuscular therapy in doses of two to five milligrams one to three times weekly controlled patients who fail to respond to oral therapy. The author believes the average therapeutic range for oral therapy is one to five milligrams per day.—*E.C.H.*

TURNER, S. J.

Bilateral microcystic degeneration of the ovaries and masculinizing syndrome. *Am. J. Obst. and Gynec.* 46: 295. 1943.

This is a case report of a 23 year-old single woman who complained of no bleeding and of growth of hair on the face and abdomen of six months' duration. Clitoris was enlarged and there was atrophy of external and internal genitalia. Resection of two-thirds. of each ovary and puncture of available cysts led to restoration of menses, growth of uterus, decrease in size of the clitoris, and cessation of excess hair growth. The ovaries showed marked luteinization of the theca and some luteinization of the ovarian stroma.—*E.C.H.*

WALSH, J. W. AND W. B. STROMME

A study of the use of diethylstilbestrol in inhibition and suppression of lactation. *Am. J. Obst. and Gynec.* 47: 655. 1944.

The patients were divided into three main groups of 50 each. Breast feeding was undesirable or contraindicated. One control group was given no therapy other than analgesics as required and the other control group was treated with breast binders, ice bags, sedatives as indicated, restriction of fluid to 1,500 cubic centimeters for three to four days and saline catharsis. The third group was given 10, 5 and 5 milligrams of diethylstilbestrol on three consecutive days immediately following delivery. Another 21 patients in whom lactation had been established were similarly treated with diethylstilbestrol. Fifteen other patients who had nursed were used as a control and either no therapy or the binder routine was used. Four patients with nonsuppurative mastisis were treated with diethylstilbestrol alone.

Diethylstilbestrol was found highly effective in preventing the development of and in minimizing the intensity of breast pain. Engorgement

was reduced in the diethylstilbestrol group to one-third that of the controls; it was less intense and developed later in the puerperium. Lactation, although frequently delayed in onset with diethylstilbestrol, was definitely, though less markedly depressed, than were the other features studied.—*E.C.H.*

WATTS, R. M. AND F. L. ADAIR

Excretion of estrogen and gonadotropin in late pregnancy. *Am. J. Obst. and Gynec.* 46: 183. 1943.

The methods used by the authors for the extraction and quantitative assay of estrogen and gonadotropin from pregnancy urine are given. The estrogen and gonadotropin excretion was studied in 74 patients, 70 of these during late pregnancy (averaging 35 weeks). Forty-three patients (11 normal, 14 hypertensive and renal disease, 13 pre-eclampsia and 5 eclampsia patients) had assays of both whereas in 14 patients only estrogen was assayed. The other 17 patients represented various other conditions complicating pregnancy.

It was found that there is a decrease in estrogen excretion and an increase in the excretion of gonadotropin in pre-eclampsia and eclampsia. In hypertensive and renal disease, the estrogen excretion is normal but the excretion of gonadotropin is increased. The authors believe that the estrogen-gonadotropin ratio is a better index of hormone values than the 24-hour excretion of either, because of extreme variability of individual values. The ratios of the excretion of estrogen to gonadotropin expressed as the Mean $\pm$S.E. for the above groups are: normal 6.38 $\pm$2.13; hypertensive and renal disease, 2.63$\pm$0.77; pre-eclampsia, 0.97$\pm$0.23; and eclampsia, 0.28$\pm$0.08.—*E.C.H.*

WATTS, R. M. AND F. L. ADAIR

Some observations on the hormonal content of ovarian cysts associated with pregnancy. *Am. J. Obst. and Gynec.* 47: 593. 1944.

The estrogen content was investigated in 30 ovarian cysts, three parovarian cysts and two other genital cysts associated with pregnancy. The gonadotropin content was studied in a few of these. The various types of cysts removed during pregnancy which contained estrogen were: corpus luteum,—five of seven; simple serous,—one of four; pseudomucinous,—one of seven; dermoid,—two of four. A papillary serous cyst contained no estrogen. Of the seven cysts removed during the post-partum periods, two simple serous, one papillary serous, and one of four pseudomucinous cysts contained estrogen. In ovarian cysts associated with pregnancy, 43.3 per cent contained estrogen. Gonadotropin was generally present.

Three parovarian cysts, one Gartner's duct cyst and one cystic fibroid assayed in association with pregnancy, all were positive for estrogen. Gonadotropin was found in the only parovarian cyst assayed and also in the cystic fibroid. In general, the amount of estrogen in cyst fluid was about that previously found in cysts of non-pregnant patients. Cysts removed during the post-partum period appear to contain more estrogen. —*E.C.H.*

WILLIAMS, W. W.

Routine order of examinations for the diagnosis of sterility. *Am. J. Obst. and Gynec.* 47: 537. 1944.

The author outlines in table form what he considers to be necessary examinations and laboratory studies for a complete sterility study.—*E.C.H.*

HYPOPHYSIS

SELYE, HANS

Atypical cell proliferation in the anterior lobe adenomas of estradiol-treated rats. *Cancer Research*, 4: 349, 1944.

The anterior lobe adenomas elicited in rats by long-continued estradiol treatment may exhibit signs of atypical cell proliferation. Such adenomas contained polynuclear giant cells and an unusually large number of mitotic figures. The cytoplasm of the giant cells was basophilic and frequently contained pigment granules or crystalloid inclusions. Metastases or other signs of malignancy were not observed.—*D.A.McG.*

SELYE, H.

Role of the hypophysis in the pathogenesis of the diseases of adaptation. *Canad. M. A. J.* 50: 426. 1944.

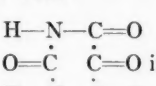
By injecting an extract of the anterior lobe of the pituitary into rats specifically sensitized to DOCA overdosage, the author noted certain pathological changes which he considers to be diseases of adaptation (nephrosclerosis, periarteritis nodosa, and cardiac changes similar to those in rheumatic fever). He suggests that these

lesions may be normally produced by chronic stress which causes the pituitary to produce an excess of an adrenotropic hormone which in turn results in the production of large quantities of DOCA or related compounds. After some time these compounds cause the pathological lesions listed above.—*P.H.S.*

PANCREAS

BRUNSCHWEIG, A., J. G. ALLEN, F. M. OWENS, JR. AND T. F. THORNTON

Alloxan in the treatment of insulin producing islet cell carcinoma of the pancreas. *J.A.M.A.* 124: 212. 1944.



Alloxan, in dogs, causes specific necrosis of the islets of Langerhans in doses of 200 mg. per kg. The authors have tried the drug in three cases of insulin producing cancer of the pancreas and report the results in one case in detail. The patient was a 32 year old man in whom extensive liver metastases had appeared after two operations for carcinoma of the pancreas. Hypoglycemic attacks occurred two to five times a day in spite of the ingestion of food every two to three hours day and night. The fasting (i.e. 4 hrs. after food) blood sugar in the 10 days of control observation averaged 16 mg.%. It was 14 mg.% just prior to an attack. In the next four days five doses of alloxan totalling 25.2 gm. were given. The fasting blood sugar during the next six days was only 21 mg.%, nevertheless attacks were milder and less frequent. There was then given 278.5 gm. in 23 days resulting in freedom from attacks for as long as 21 days at a time and blood sugars as high as 55 mg.%. The patient subsequently died following operation.—*A.P.F.*

CEBALLOS, A. AND S. ROSENBLATT

Hyperinsulinism, caused by adenoma of the islands of Langerhans (a clinical case with operative confirmation and cure by enucleation) *Prensa med. argent.*, 30: 689. 1943.

A tentative diagnosis of adenoma of the pancreas was made and surgical intervention was decided upon in view of the failure of adequate medical treatment. The tumor weighed 1.5 gm. and had a capsule on which small particles of normal pancreatic tissue were demonstrable. Histological examination led to a diagnosis of adenoma of the insular cells.

The patient was completely free of her former disturbances and there was no relapse during an observation period of four months, although the patient was exposed to prolonged periods of fasting. She was able to do her work and her menstruation became normal. Glucose tolerance tests, however, showed an abnormal curve.—*Courtesy Diabetes Abstracts.*

FEINBLATT, H. M., B. B. ALPERT, AND E. A. FERGUSON.

Juvenile diabetes insulin sensitivity. *L. Lab. & Clin. Med.*, 29. 366. 1944.

The authors present experimental and clinical evidence to the effect that the diabetogenic factor of the anterior pituitary gland plays an important role in the production of insulin sensitivity. Eight out of 10 mice survived the administration of lethal doses of insulin when previously injected with "anterior pituitary diabetogenic factor" prepared so that 1 cc. was equivalent to 1 gm. of the original gland substance. Three injections of 0.25 cc. each were given 24, 12, and two hours prior to the insulin injection. On the contrary, nine of 10 mice died when given the same dose of insulin without pretreatment. The findings in 11 patients with "juvenile diabetes" were summarized. The first six were instances of glycosuria with normal fasting blood sugars, a not too rare event in any diabetic clinic, The authors believe, however, that these patients represented a distinct group in each of whom some factor, such as puberty, infection, menstrual disorder, nausea and vomiting, and so forth, had altered the activity of the anterior pituitary in such a way as to influence the release of the diabetogenic factor. It was felt that sudden increases in glucose tolerance have been associated with a decrease in pituitary diabetogenic activity, and conversely an increased glycosuria implied an over-production of the diabetogenic factor as a result of one of the forms of physical or emotional strain above mentioned. The remaining five cases illustrated the decreased tolerance for glucose which accompanied the overactivity of the anterior pituitary during pregnancy, and the dangers of the unrecognized sudden increase in tolerance which is to be observed immediately post-partum. The authors ascribe this post-partum insulin sensitivity to an anti-anterior lobe substance which may originate in the posterior pituitary.—*T.H.McG.*

FOSTER, DANIEL P., WM. L. LOWRIE AND J. MACMILLAN

A plan of treatment for diabetic acidosis. *Am. J. Digest. Dis.*, 10: 371. 1943.

The treatment of diabetic acidosis requires hospitalization in order to re-establish normal quantitative values for the chemically altered body fluids. Carbohydrate should be administered as soon as the blood-sugar estimations reveal a decided decline. Hypoglycemia should be avoided. Dehydration should be combated vigorously with salt solution. Patients utilize, with benefit, 5,000 to 8,000 cc. of fluid in the first twenty-four hours. A detailed tabulation of initial orders is outlined, followed by carrying-orders to be adopted when the acidosis is corrected.

A statistical study of eighty-three instances of diabetic acidosis is presented. These cases are divided into two groups: Group I, consisting of patients treated before 1931; and Group II, consisting of those patients treated in 1937, 1938, and 1939.

The average age of the two groups remained unchanged, as did the sex ratio. Underweight diabetics were more frequently affected. Infection heads the list as the most common cause of acidosis, and dietary errors occupy second place. Insulin errors seem to be becoming a more frequent cause. Patients still omit insulin under the influence of misleading advertisements.

The mortality rate, if corrected to include only those cases in which acidosis was the chief factor, was 2 per cent in Group I and 3.1 per cent in Group II. A tabulation of the other causes of death is presented.—*Courtesy Diabetes Abstracts.*